Citation for published version:
Baker, DR & Kasprzyk-Hordern, B 2011, 'Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry', Journal of Chromatography A, vol. 1218, no. 44, pp. 7901-7913. https://doi.org/10.1016/j.chroma.2011.08.092

DOI:
10.1016/j.chroma.2011.08.092

Publication date:
2011

Document Version
Peer reviewed version

Link to publication

NOTICE: this is the author's version of a work that was accepted for publication in Journal of Chromatography A. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Journal of Chromatography A, Vol 1218, Issue 44, (2011), DOI 10.1016/j.chroma.2011.08.092

University of Bath

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 13. May. 2019

Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry

David R. Baker[1] *and Barbara Kasprzyk-Hordern*[2*]

[1]University of Huddersfield, Department of Chemical and Biological Sciences, School of Applied Sciences, Queensgate, Huddersfield HD1 3DH, UK

[2] University of Bath, Department of Chemistry, Faculty of Science, Bath BA2 7AY, UK

ABSTRACT

Presented is the first comprehensive study of drugs of abuse on suspended particulate matter (SPM) in wastewater. Analysis of SPM is crucial to prevent the under-reporting of the levels of analyte that may be present in wastewater. Analytical methods to date analyse the aqueous part of wastewater samples only, removing SPM through the use of filtration or centrifugation. The development of an analytical method to determine 60 compounds on SPM using a combination of pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry (PLE-SPE-LC-MS/MS) is reported. The range of compounds monitored included stimulants, opioid and morphine derivatives, benzodiazepines, antidepressants, dissociative anaesthetics, drug precursors, and their metabolites. The method was successfully validated (parameters studied: linearity and range, recovery, accuracy, reproducibility, repeatability, matrix effects, and limits of detection and quantification). The developed methodology was applied to SPM samples collected at three wastewater treatment plants in the UK. The average proportion of analyte on SPM as opposed to in the aqueous phase was < 5 % for several compounds including cocaine, benzoylecgonine, MDMA, and ketamine; whereas the proportion was >10 % with regards to methadone, EDDP, EMDP, BZP, fentanyl, nortramadol, norpropoxyphene, sildenafil and all antidepressants (dosulepin, amitriptyline, nortriptyline, fluoxetine and norfluoxetine). Consequently, the lack of SPM analysis in wastewater sampling protocol could lead to the under-reporting of the measured concentration of some compounds.

Keywords: Suspended solids, suspended particulate matter, solid phase extraction, SPE pressurized liquid extraction, PLE, LC-MS/MS, wastewater, illicit drugs, pharmaceuticals, sewage forensics, sewage epidemiology

* Corresponding author: E-mail: b.kasprzyk-hordern@bath.ac.uk; Fax: +44(0) 1225 386231; Tel: +44 (0) 1225 385013

1 INTRODUCTION

Recent publications have demonstrated the presence of drugs of abuse and associated metabolites in the aquatic environment [1-10]. Subsequently, the measured analyte concentration is often used to back-calculate drug usage in local communities (so called sewage forensics or sewage epidemiology) or to assess the environmental risk posed to humans and wildlife. With regards to the analysis of wastewater, all published procedures for the analysis of drugs of abuse remove SPM through filtration or centrifugation, and analyse the aqueous part of the sample only. However, detailed investigations into the amount of compounds sorbed to suspended particulate matter (SPM) are still missing. Without an understanding of the amount of compound sorbed onto SPM and the effect of filtration, there is potential in the case of some compounds to significantly underestimate the total concentration of drug residue in the studied environmental sample.

Only one limited study has been published to date for the analysis of drugs of abuse on SPM by Metcalfe et al. [6]. The study reported the use of ultra-sonication (USE) of SPM to assess the levels of methamphetamine, MDA, MDMA, cocaine and benzoylecgonine. This study is limited due to the analysis of only two samples in singlet and the relatively small number of compounds monitored. While methodologies for the analysis of SPM are lacking, many methods have been published for the analysis of soils, sludge and sediment for the presence of pharmaceuticals, although the majority of these methods are developed for a small range of compounds that are chemically related [11,12]. Kaleta et al. [13] analysed sewage sludge using USE for the presence of amphetamine only. Only a few methods have been published with regards to the multi-class analysis of pharmaceuticals in solid environmental samples. The main parameters of these multi residue methods are summarised in Table S1, along with the USE methods developed by Metcalfe et al. [6] and Kaleta et al. [13].

Typically, methods for the extraction of environmental solids are based on some form of sample drying through either air-drying in the dark [14-16], heating in an oven [17], or (most commonly) lyophilisation [18-22]. Extraction is generally carried out through the usage of pressurised liquid extraction (PLE). PLE is a well-established analytical technique that has been used to extract a range of different compounds from numerous different matrices [11,12,23]. PLE offers short extraction times, low solvent consumption and provides additional extraction by adding an inert material to the extraction cell [11]. Post-treatment of extracts typically involves solid phase extraction (SPE) followed by analysis using LC-MS/MS [16,20-22].

An analytical methodology to study drugs of abuse on SPM is critical to prevent under-reporting of target analytes in wastewater samples. To date only one very limited study has been published for this purpose [6]. As a result, there were three major aims of this study:

1) To develop the first multi-residue method for the analysis of important illicit drugs and pharmaceuticals on SPM from several classes of compounds including: stimulants, opioid and morphine derivatives, benzodiazepines, antidepressants, dissociative anaesthetics, drug precursors, human urine indicators and their metabolites. The post extraction of PLE extracts was based on the SPE and LC-MS/MS developed, as previously reported by this group [24]. The aim was to develop a relatively straightforward and efficient method based on PLE-SPE-LC-MS/MS with a single extraction procedure for each of the techniques.

2) To apply the new method to collect data on the occurrence of the selected analytes on SPM in raw wastewater.

3) Based on data obtained, to assess whether the filtration of wastewater is likely to result in significant underreporting of the concentration of analyte in samples.

2 EXPERIMENTAL

2.1 Chemicals and materials

Analyte names, CAS number, molecular formula, log K_{ow}, log D_{ow}, pK_a and supplier are shown in Table S2. Surrogate/internal standards were all purchased from LGC, with the exception of caffeine-d9 (Sigma-Aldrich). All standards and internal standards were of the highest purity available (>97%). Individual stock solutions were purchased or prepared from powdered substance in either acetone or methanol at a concentration of 1 or 0.1 g L^{-1} and stored in the dark at −20 ◦C. Mixed standard solutions were prepared at 10 mg L^{-1} in methanol and diluted as necessary to prepare working solutions. LC-MS mobile phase solvents and additives were all of LC–MS quality and purchased from Sigma–Aldrich, with the exception of H_2O which was purchased from Fisher. Hydrochloric acid (37%), ammonium hydroxide (30%), and 5% dimethylchlorosilane (DMDCS) in toluene were purchased from Sigma–Aldrich. HPLC grade methanol, glacial acetic acid (>99%) and analytical grade sand were purchased from Fisher. Ultrapure water used for PLE was taken from a Barnstead Nanopure water purification system (Thermoscientific, UK) with a specific resistance of 18 MΩ-cm.

Solid phase extraction (SPE) was carried out with Gilson SPE, Aspec XL4 (Anachem, UK). Oasis 60 mg MCX and 60 mg HLB cartridges were purchased from Waters (Waters, UK). SPE samples were eluted into borosilicate glass tubes (12mm x 75mm, Fisher, UK) and evaporated with a TurboVap LV concentration workstation (Caliper, UK). All glassware used was silanised by rinsing (once) with DMDCS for 15 seconds, toluene (twice) and finally methanol (thrice).

Soil and SPM samples were extracted by PLE using an ASE 150 system (Dionex, UK). Whatman glass fibre filters were placed at the bottom of 100 mL extraction cells to prevent possible blockage of the end cap. Surrogate/internal standards were spiked into each sample at the following concentrations: amphetamine-d11 (150 ng g^{-1}), methamphetamine-d14 (100 ng g^{-1}), nicotine-d4 (87.5 ng g^{-1}), buprenorphine-d4 (100 ng g^{-1}), diazepam-d5 (100 ng g^{-1}), heroin-d9 (300 ng g^{-1}), cocaine-d3(100 ng g^{-1}), fentanyl-d5 (100 ng g^{-1}), codeine-d6 (100 ng g^{-1}), ketamine-d4 (100 ng g^{-1}), fluoxetine-d6 (200 ng g^{-1}), propoxyphene-d11 (100 ng g^{-1}), oxycodone-d6 (100 ng g^{-1}), norpropoxyphene-d5 (200 ng g^{-1}), MDMA-d5 (100 ng g^{-1}), oxazepam-d5 (100 ng g^{-1}), mescaline-d9 (125 ng g^{-1}), PCP-d5 (100 ng g^{-1}), morphine-d6 (300 ng g^{-1}), benzoylecgonine-d8 (100 ng g^{-1}), LSD-d3 (100 ng g^{-1}), methadone-d9 (100 ng g^{-1}), EDDP-d3 (100 ng g^{-1}), methaqualone-d7 (100 ng g^{-1}), dihydrocodeine-d6 (100 ng g^{-1}), MBDB-d5 (100 ng g^{-1}), cocaethylene-d8 (100 ng g^{-1}), MDEA-d5 (100 ng g^{-1}), temazepam-d5 (55 ng g^{-1}), caffeine-d9 (600 ng g^{-1}) and MDA-d5 (100 ng g^{-1}).

2.2 Sample collection and preparation

Large volume wastewater grab samples (approx. 8 litres) were collected from three major wastewater treatments plants (WWTPs) in the UK, once in October and once in November 2011. The three WWTPs served populations in excess of 100,000 inhabitants. Samples were collected after primary screening and before any secondary treatment. All samples were collected in amber silanised bottles with Teflon faced caps (Fisher, UK). Bottles were transported back to the laboratory in a dark and iced coolbox. Wastewater samples were stored in the dark at 4 °C for no longer than 18 hours before being processed. All samples were analysed in duplicate.

A small portion of the wastewater (around 400 mL) was separated from the bulk sample and filtered through GF/D 2.7 µm glass fibre filters (Whatman, UK) and subsequently through GF/F 0.7 µm glass fibre filters (Whatman, UK). After filtration, samples were acidified with HCl to pH 1.8 and spiked with internal standards. Samples were extracted with SPE as described in section 2.4.

SPM was removed from the bulk wastewater sample using a combination of centrifugation and filtration. Bottles used during centrifugation and glass microfibre filters were all oven-dried (40 °C) before use to a constant weight. Wastewater was centrifuged at 13,000 rpm (25,931 x g), 10 °C, 18 minutes, with the supernatant decanted off and vacuum filtered through GF/D 2.7 µm glass fibre filters (Whatman, UK). Once empty, collection bottles were rinsed with a small amount of ultra pure water and filtered to account for any remaining residues inside the collection bottles. Centrifuged extracted solids, in addition to the filters and bottles used during centrifugation, were oven-dried at 40 °C for approximately 10 hours, until a constant weight was achieved. The final dry weight of solids was calculated by taking into account the weight of the centrifuged extracted solids, and the final weight of the centrifugation bottles and filters (minus their original oven-dried weights before use). Samples were subsequently homogenised and reduced in particle size through the use of a pestle and mortar, before being stored in the dark at -18 °C until extraction. Grab samples of soil collected from the University of Huddersfield grounds were used for analytical method development and validation, after being oven dried at 40 °C for 12 hours and finely ground using a pestle and mortar. Soil was used for method development as this matrix, as opposed to SPM, was free of target analytes and easily obtainable.

2.3 Pressurized liquid extraction

The PLE method was optimised through selection of extraction solvent, extraction temperature and number of cycles. Each parameter was investigated in series using initial conditions with regards to PLE as follows: temperature, 80 °C; preheat period, 5 min; static cycles, 3; static time, 5 min; flush volume, 60%; purge time,

250 s; pressure, 1,500 psi and extraction cell, 100 mL. Solvents investigated during optimisation were methanol/water (1/1, 1/2, 1/3, 2/1, 2/1, v/v) all adjusted to pH 2 with acetic acid. Temperatures of 40, 60, 80, 100 and 120 °C were evaluated using the optimised solvent. The number of extraction cycles required was optimised by collecting 4 individual extraction cycles of the same sample. During method development, 1 g of soil was spiked with 150 ng g^{-1} of each compound, with recoveries calculated against spiked matrix after PLE (before SPE). The final PLE method was as follows: temperature, 80 °C; preheat period, 5 min; static cycles, 3; static time, 5 min; flush volume, 60%; purge time, 250 s; pressure, 1,500 psi. Three PLE rinse cycles were performed before starting PLE analysis and one rinse was carried out between each subsequent sample.

The extract obtained in PLE (approximately 114 mL) was poured into a 500 mL volumetric. The bottle used to collect the PLE extract was then rinsed three times (approximately 300 mL in total) with ultrapure water (pH 1.8,. adjusted with HCl). The sample was then made up to 500 mL with ultrapure water (pH 1.8) and extracted by SPE in the same manner as described in section 2.4. An exception to this was during the investigation into extraction solvent, in which samples were diluted to 1000 mL to ensure that the varying amounts of methanol in the extract were sufficiently diluted to negate the effects on SPE recovery.

2.4 Solid phase extraction

SPE was carried out with the usage of Oasis MCX cartridges. Conditioning was performed with MeOH (2 mL) and equilibration with 2% $HCOOH/H_2O$ (2mL, pH 2), both at a flow rate of 3 mL min^{-1}. Acidified (pH 1.8) PLE samples (500 mL) or aqueous wastewater samples (100 mL) were passed through the MCX cartridge at a rate of 6 mL min^{-1}. Immediately following loading, cartridges were washed with 2 % $HCOOH/H_2O$ (2 mL, pH 1.8) at a flow rate of 3 mL min^{-1} and subsequently wrapped in aluminium foil and stored at −20 ˚C no longer than one week before being eluted. Cartridges were washed with 0.6 % HCOOH/MeOH (2 mL, pH 1.8) at a flow rate of 3 mL min^{-1} followed by elution with 7 % NH_4OH/MeOH (3 mL) at a flow rate of 1 mL min^{-1} into silanised vials. Extracts were evaporated to dryness (40 ˚C, N_2, 2–10 psi) and reconstituted with 0.3 % CH_3COOH/5 % $MeOH/H_2O$ (v/v) (500 µL). All samples were filtered through 0.2 µm PTFE filters (Whatman, Puradisc, 13mm) before being transferred to maximum recovery deactivated vials with PTFE septa (Waters, UK).

2.5 LC-MS/MS

The aforementioned drug residues and associated metabolites were measured with a fully validated, highly selective and sensitive LC-MS/MS method [24]. Briefly, separation was achieved with the usage of Waters ACQUITY UPLC™ system (Waters, UK) consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager. Analytes were analysed with an AQUITY UPLC BEH C18 (1.7 µm; 1mm × 150 mm) column, with a mobile phase consisting of mobile phase A (pH 2.9): 79.7%H_2O, 20%MeOH, 0.3%CH_3COOH and mobile phase B (pH 3.3): 99.7%MeOH, 0.3%CH_3COOH at a flow rate of 0.04 mL min^{-1} and a temperature of 30 °C. The gradient programme was as follows: 0min – 100% A, 17 min – 41.3% A, 17.2 min – 0% A, 20.2 min – 0% A, 20.3 min – 100% A, 34.0 min – 100% A. An injection volume of 20 µL was used.

A triple quadrupole mass spectrometer (TQD, waters, UK) equipped with an electrospray ionisation source was used for the quantification of target analytes. The analyses were performed in positive mode. The mass spectrometer was operated in selected reaction monitoring (SRM) mode, measuring the fragmentation of the protonated pseudo-molecular ions of each compound. Masslynx 4.1 software (Waters, UK) was used to collect and analyse all data.

2.6 Quantification and confirmation

Each compound was quantified by MRM, with the protonated molecular ion employed as the precursor. The most abundant transition product ion was typically used for quantification; with a second transition, for nearly all compounds, used for confirmation (see Table S3). The criteria to confirm the presence of analyte in environmental samples included ensuring the ratio of the retention time of internal standard/analyte in the standard sample was within 2.5 % of the ratio of internal standard/analyte in the 'test' sample. Furthermore, ensuring the ion ratio of the quantifier ion to that of the qualifier ion was within tolerance ranges as described by the EU guidelines [25]. 31 deuterated internal standards were used to compensate for signal suppression or enhancement of analytes in the ESI source and low SPE or PLE recoveries. A deuterated internal standard for all analytes was not possible due to lack of commercial availability; hence an internal standard that was similar in structure and gave similar analytical responses was selected as a surrogate for those compounds.

The percentage of analyte on SPM (P_{SPM}) was experimentally determined using Equation 1:

$$P_{SPM} = \frac{\left(\frac{C_{SPM}\ x\ M_{SB}}{V_W}\right)}{\left(\frac{C_{SPM}\ x\ M_{SB}}{V_W}\right) + C_{DISS}} x100$$

Equation 1 – Experimentally determined percentage of analyte sorbed onto SPM

Where: C_{SPM} is the concentration of analyte determined on SPM (g g^{-1}); M_{SB} is the dry weight of SPM in the wastewater sample (g L^{-1}); V_W is the volume of wastewater sample (L); C_{DISS} is the concentration of analyte in the aqueous phase of the sample (ng L^{-1}).

2.7 Method validation

The performance of the method was evaluated through estimation of linearity and range, recovery, accuracy, reproducibility, repeatability, matrix effects, and limits of detection and quantification. All results expressed as ng g^{-1} are done so on a dry-weight basis.

Linearity was investigated over a ten-point calibration with spiked soil samples ranging from 0.5 – 500 ng g^{-1} analysed in duplicate. The calibration curve was prepared by calculating the ratios between the peak area of each substance and the peak area of the internal standard. Masslynx 4.1 software was used to analyse and process all data. Acceptable linearity was obtained with a correlation coefficient >0.99 with ≥5 data points. Overall method repeatability was evaluated by spiking soil samples with 50 ng g^{-1} of each compound (n = 5). Overall method reproducibility was evaluated at 50 ng g^{-1} over a three day period (n = 2). Accuracy of the method was assessed as the percentage deviation from the known amount of analyte added to the sample at one concentration level, 50 ng g^{-1}.

Recoveries regarding the PLE procedure and the overall PLE-SPE-LC-MS/MS were evaluated by spiking both soil and SPM at 50 ng g^{-1}. To calculate the PLE recovery, peak areas of analytes in soil/SPM spiked before PLE were compared to peak areas of analytes in soil/SPM extract spiked after PLE but before SPE. To calculate PLE-SPE-LC-MS/MS recovery, peak areas of analytes in soil/SPM spiked before PLE were compared to peak areas of analytes in soil/SPM extract spiked during reconstitution step (after SPE). The recovery for the SPE-LC-MS/MS procedure was calculated by spiking soil/SPM after PLE (but before SPE) and comparing peak areas of analytes in soil/SPM spiked after PLE with peak areas in soil/SPM extract spiked during reconstitution. Thus each recovery is decoupled from matrix effects. Matrix effects are presented separately and were determined for each compound as a percentage decrease in peak area of analyte in sample matrix (minus peak area present in blank sample) compared to sample diluent.

LC-MS/MS instrumental detection limits (IDL) and quantification limits (IQL) were experimentally determined using signal-to-noise approach through analysis of a series of low concentration standards (in spiked sample diluent) as previously reported [24]. IDL was determined at the lowest concentration that provided S/N ≥ 3 for transition 1. IQL limits were determined at the lowest concentration that provided S/N ≥ 10 for transition 1 and S/N ≥ 3 for transition 2. PLE-SPE-LC-MS/MS method detection limits (MDL) and method quantification limits (MQL) were determined using soil spiked with known concentrations of analytes and then extracted according to the procedure described above. MQLs were determined both experimentally with the usage of signal-to-noise approach ($MDL_{S/N}$, $MQL_{S/N}$) and calculated with the usage of Eqs 2 and 3 (MDL_{calc}, MQL_{calc}). To estimate MDL_{calc} and MQL_{calc} the IDL and IQL was taken into consideration in addition to the total method recovery (including matrix effects) and the sample concentration factor involved. To clarify, this process firstly involved estimating the instrumental limits for solid samples as opposed to liquid. For instance, if the IQL for a certain analyte was 100 ng L^{-1} (the equivalent of 50 ng 500 mL^{-1}), and as the solid sample (1 g) is diluted in 500 mL, this allows an instrumental quantification limit of 50 ng g^{-1} to be estimated for the solid sample (termed S.IQL) (assuming no loss of analyte at any step in the procedure or no concentration factor at this point). Subsequently, once a S.IQL or S.IDL has been estimated for the analyte, Equation 2 and Equation 3 may be used to take into consideration total method recovery (that includes matrix effects) and the concentration factor.

$$\mathrm{MDL_{Calc}} = \frac{\mathrm{S.IDL\ x\ 100}}{\mathrm{T.Rec\ x\ CF}}$$

Equation 2 – PLE-SPE-LC-MS/MS method detection limit calculation

$$\mathrm{MQL_{Calc}} = \frac{\mathrm{S.\,IQL\ x\ 100}}{\mathrm{T.\,Rec\ x\ CF}}$$

Equation 3 - PLE-SPE-LC-MS/MS method quantification limit calculation

Where: S.IDL is the instrumental detection limit (ng g^{-1}) (see above for discussion), S.IQL is the instrumental quantification limit (ng g^{-1}), T.Rec is the total PLE-SPE-LC-MS/MS recovery (%) and CF is the concentration factor, which in this method denotes 1000.

Spiking was carried out by loading the soil (1 g) or SPM (1 g) into an extraction cell partially filled with sand and spiking with a small volume of internal standards in methanol (10 µL) (and analytes in the case of recovery experiments). The extraction cell was then placed in a fume cupboard for 20 minutes to allow the solvent to evaporate and the remaining cell filled with sand.

3 RESULTS AND DISCUSSION

3.1 Method development

To achieve a fast and efficient extraction procedure, the PLE variables (solvent, temperature and extraction cycles) were evaluated. Each PLE parameter was investigated in a step-wise manner using the initial conditions described in section 2.3. Initial conditions were selected based on a literature review, with the most relevant methods to this study summarised in Table S1. The variety of compounds studied exhibited Log D values in the range -2.3 – 4.7 at pH 7 and -1.9 -5.4 at pH 8 (predicted using ACD labs software [26]).

The PLE method was developed based on the SPE and LC-MS/MS procedure described previously [24]. The SPE method was optimised by evaluating the percentage of organic solvent that a sample can contain before recovery is adversely affected. This parameter is important due to the need to dilute PLE extracts to reduce organic composition; generally so organic content is around 5 % or less [20,27]. It was found with the use a mixed mode SPE sorbent that methanol content may be up to 25 %, thereby significantly reducing the required dilution of PLE extracts and in turn the amount of time required for extraction. This study is described in more detail in the supplementary material.

3.1.1 Solvent

As with the majority of solid-liquid extraction techniques, solvent is one of the most important parameters to optimise in the development of an efficient extraction procedure [11]. Due to the range of compounds selected in this study being characterised by widely differing polarities, a solvent with some organic content in water was likely to provide the best recoveries. Consequently, various different solvents were tested containing varying amounts of methanol/water (1/1, 1/2, 1/3, 2/1, 2/1, v/v) with all solvents adjusted to pH 2 with acetic acid. The combination of methanol/water, as opposed to other solvents such as acetonitrile/water, was selected based on published literature identifying this mixture as providing higher recoveries in the majority of extraction methods [16,18,20,21]. It was decided to test the solvents at an acidic pH due to authors [18,28,29] reporting the highest recoveries with an acidic solvent.

To optimise the solvent, soil was directly spiked with 150 ng g^{-1} of each compound and extracted with methanol/water (pH 2) at different ratios. Absolute recoveries were calculated against soil that was spiked after PLE, but before SPE. Thus, recoveries are a reflection of PLE recovery decoupled from matrix effects. Recoveries are shown in Table S5 with the highest recovery obtained for each compound highlighted.

As was expected with multi-residue analysis, the results did not show one particular solvent to provide the highest recoveries for all compounds. Some analytes reported very little change in all solvents tested, including cocaine, benzoylecgonine, cocaethylene, MDA, MDMA, 6-acetylmorphine, ketamine and norketamine. In comparison, some compounds showed much higher recoveries in certain solvents. EDDP reported a recovery of 82 % with methanol/water at 1/1 (v/v), as opposed to a recovery of 52 % at 3/1 and 65 % at 1/3. Methadone also provided a similar recovery pattern to that of its main metabolite EDDP. All of the amphetamine type compounds provided highest recoveries when extracted in the solvent containing the highest percentage of water. The antidepressants all reported highest recoveries when extracted using methanol water, 1/1. For instance fluoxetine achieved a recovery of 84 % in methanol/water, 1/1 (v/v), whereas a recovery of 23 % was obtained at 1/3 and 67 % at 3/1. Similarly, the benzodiazepines reported optimal recoveries in the same

solvent, with exception of temazepam and oxazepam that reported higher recoveries with an increased amount of methanol. Recoveries were < 30 % in all solvents tested for fentanyl, BZP, PCP and 7-aminonitrazepam. As a compromise between the optimal recoveries for all compounds, a solvent containing methanol/water, 1/1 (v/v) was selected for further development.

3.1.2 Temperature

Application of higher temperatures in PLE decreases the viscosity of solvents, therefore allowing better penetration into sample matrix [27]. Furthermore, higher temperatures increase diffusion rates and increase the ability of the solvent to disrupt matrix-analyte interactions. Consequently, the release of analytes from active sites in the matrix is speeded up, which is considered the rate-limiting step in many environmental applications [23]. Low temperature can decrease recoveries. However, on the other hand, too high a temperature can also decrease recoveries. This may be due to thermal degradation of analytes or loss of selectivity in the method that leads to the more efficient release of interfering matrix components [11,20,28]. Temperatures of 50 to 110 °C were studied by Vazquez-Roig et al. [22] and found increasing recoveries up to 90 °C. Over this temperature the recovery of some pharmaceuticals decreased. Similarly, Barron et al. [20] studied a temperature range of 40 to 120 °C and selected the optimum temperature of 60 °C due to loss of some compounds over this temperature.

In this study, temperatures of 40, 60, 80, 100 and 120 °C were studied using the optimised solvent methanol/water, 1/1 (v/v) at pH 2. Soil was directly spiked with 150 ng g^{-1} of each compound. Absolute recoveries were calculated in the same manner as previously undertaken during the solvent investigation. Absolute recoveries were calculated against soil that was spiked after PLE, but before SPE; hence recoveries are a reflection of PLE recovery decoupled from matrix effects. Recovery values for all analytes at each temperature are listed in Table S6.

The increase in temperature resulted in changes in recovery for a number of compounds. For the majority of compounds an increase in recovery was observed as the temperature increased from 40 °C. However, for some compounds after a certain temperature a decrease in recovery was observed. The negative effect of an increase in temperature is shown in Figure S1a, with a decrease in recovery observed after 60 - 80 °C. As previously mentioned, this may be due to thermal degradation of analytes or loss of selectivity that occurred as a result of a less selective method extracting more interfering matrix components [11,20,28]. In contrast, the trend observed for certain compounds showed a continual increase in recovery with temperature (see Figure S1b). The increase in recovery with temperature was especially pronounced for PCP, venlafaxine and EDDP. For instance, at 40 °C venlafaxine reported a recovery of 20 %, while at 120 °C a recovery of 95 % was observed. A temperature of 80 °C was selected for further development as a compromise between the optimum recoveries for the studied compounds.

3.1.3 Extraction cycles

The number of cycles employed in PLE is important as the introduction of fresh solvent maintains a suitable solvent-to-sample equilibrium, and improves partitioning into the liquid phase [11,23]. A static time of 5 minutes was selected based on the vast majority of authors reporting 5 minutes as the optimal static period [16,20,21,27]. To investigate the number of extraction cycles required soil was spiked with 150 ng g^{-1} of each compound. Four successive extractions were carried out on the same sample with each cycle collected and analysed separately. Results shown in Figure S2 are expressed as cumulative absolute recovery.

Absolute recovery was calculated by comparison of peaks areas to a sample of blank soil spiked after PLE, but before SPE.

It was found that the first and second cycle contained the highest concentrations of each analyte with only a small fraction of each compound detected in the third cycle, and negligible amounts of each analyte detected in the fourth cycle. For this reason three cycles were selected as optimum.

3.2 Method validation

The performance of the method was evaluated through estimation of linearity and range, recovery, accuracy, reproducibility, repeatability, matrix effects, and limits of detection and quantification. All method performance data is listed in Table 1 and recoveries in Table 2. LC-MS/MS chromatograms for all analytes spiked on SPM before PLE at 50 ng g^{-1} are shown in Figure S3.

Soil samples were spiked in the concentration range 0.5 - 500 ng g^{-1} and calibration curves generated as described in section 2.7. The range was selected based on expected concentrations on SPM in wastewater samples. Acceptable linearity was considered $R^2 \geq 0.99$ with a minimum of n = 5 data points. The correlation coefficient for all compounds was $R^2 > 0.992$ with the majority of compounds achieving linearity of $R^2 > 0.997$ (Table 1). An exception to this was fentanyl ($R^2 = 0.979$). The range for the majority of compounds incorporated all ten data points between the range 0.5 - 500 ng g^{-1}. Sensitivity for some compounds was above 0.5 ng g^{-1} (12 in total: ecgonidine, BZP, BDB, mescaline, oxymorphone, norpropoxyphene, nortramadol, temazepam, nitrazepam, norfluoxetine, sildenafil and norephedrine), and so was measured at a higher starting concentration. The upper concentration range was < 250 ng g^{-1} for a small number of compounds (6 in total: anhydroecgonine methyl ester, ecgonidine, TFMPP, BDB, oxymorphone and methaqualone), which could be due to several factors such as SPE breakthrough or the concentration being above the linear range of the mass spectrometer. Nevertheless, with a minimum of n = 5 data points, the calibration curve was considered acceptable for quantification purposes.

MDLs and MQLs were estimated based on a signal to noise approach as described in section 2.7. MQLs obtained in soil were < 1.0 ng g^{-1} for 56 compounds and on SPM < 2.6 ng g^{-1} for 54 compounds (Table 1). This method therefore offers excellent sensitivity for the quantification of multi-class compounds at trace levels. High limits of quantification for a small percentage of compounds were reported. This was a result of low PLE recovery (BZP, fentanyl), low SPE recovery (anhydroecgonine methyl ester, ecgonidine) or high ion suppression when extracted from SPM (diazepam, nordiazepam, and mescaline).

Repeatability was assessed at a concentration of 50 ng g^{-1} with n = 5 samples, with reproducibility conducted at the same concentration over a three day period. Precision < 20 % was considered acceptable due to the number of analytical steps involved in the procedure. Repeatability was determined to be ≤ 10 % for nearly all compounds (56 analytes), whilst on the other hand a small number of compounds reported values > 20 % including fentanyl (31 %), fluoxetine (37 %) and norfluoxetine (35 %). Reproducibility was < 15 % for 55 compounds, with values > 20 % for analytes BZP (42 %), fluoxetine (48 %) and norfluoxetine (52 %). All compounds reported an accuracy ± 18 % that was considered adequate for environmental application.

Recovery was determined by spiking soil and SPM at 50 ng g^{-1} for each analyte. Recoveries were calculated as described in section 2.7. Absolute recoveries were determined in relation to 1) the PLE method, 2) the SPE method (including evaporation and reconstitution), and 3) the full PLE-SPE-LC-MS/MS method. Relative recovery was determined for the full procedure and all recoveries are listed in Table 2. PLE-SPE-LC-MS/MS recoveries for the majority of compounds (38 in total) were ≥ 60 % in soil, and

similarly in SPM recoveries were ≥ 60 % for most analytes (34 in total). By providing recoveries for the SPE and PLE procedure separately, observations as to why low recoveries have been reported can be extracted. For instance, temazepam in both soil and SPM reported a PLE recovery around 90 %, whereas the SPE recovery was around 10 %, resulting in a low PLE-SPE-LC-MS/MS recovery. The use of deuterated internal standards to compensate for sample preparation and matrix effects provided recoveries of around 100 % for all compounds, with a small number of exceptions. When analysed on SPM, low relative recoveries were obtained for anhydroecgonine methyl ester (11 %), ecgonidine (7 %) and methcathinone (20 %).

MS signal suppression or enhancement was investigated by spiking soil/SPM at 100 μg L^{-1} during reconstitution and comparing peak areas to that of sample diluent spiked at the same concentration. The results are listed in Table 1, with a value of 0 % indicating no matrix effect, > 0 % indicating signal suppression and a value < 0 % indicating signal enhancement. For the majority of compounds in soil, a small amount of enhancement or suppression of 20 % or less was observed. Surprisingly high signal enhancement was observed in soil for nortramadol (118 %) and norephedrine (212 %). Matrix effects in SPM were more pronounced. Although matrix effects were different for every compound, many compounds observed signal suppression in the range of 20 - 50 %. The highest signal suppression was observed for nordiazepam at 97 %, with the parent compound diazepam also high at 87 %. Signal enhancement was again observed for norephedrine (363 %) and tramadol (300 %). In addition, significant enhancement was observed for amphetamine (118 %), propoxyphene (191 %), norfluoxetine (165 %) and ephedrine (123 %). Radjenovic et al. [21] also observed extremely high signal suppression in sewage sludge, albeit with a different set of compounds, with enhancement of: acetaminophen 121.7 – 311.1 %, propyphenazone 246 – 375.7 %, sulfamethoxazole 272 – 361.1 %, glibenclamide 177.1 – 384.4 % and ofloxacin 626.7 – 669.4 %.

4 Application to samples from the UK

4.1 Results

The developed methodology was applied to the analysis of SPM extracted from wastewater collected from three major WWTPs in the UK. Of the SPM samples analysed, 31 compounds were determined at a concentration >MQL, with a further three compounds detected at a concentration MDL> < MQL. The concentration of analyte measured on SPM and dissolved in the aqueous phase is listed in Table 3. This table also reports the percentage of the total concentration that was measured on SPM.

The adsorption of cocaine to SPM ranged from 1.8 – 2.7 ng g^{-1}, which represented a proportion on SPM ranging from 0.9 – 1.8 %. To a lesser extent, benzoylecgonine was determined on SPM as a proportion ranging from 0.02 to 0.2 % and cocaethylene 1.4 – 2.2 %. Amphetamine was detected in four samples. However, the ion ratio of the two transitions for this compound was outside the permitted tolerance range and did allow reliable quantification. Nevertheless this compound was presented in the results for information purposes. Amphetamine presented a proportion on SPM ranging from 1.6 – 8.6 %. Methamphetamine was not detected in wastewater or sorbed to SPM.

Levels of methadone and EDDP detected on SPM were found to be important. The concentration of methadone ranged from 19.4 - 57.6 ng g^{-1}, resulting in a proportion on SPM of 8.1 -18.6 %. EDDP concentration was between 30.1 – 194.0 ng g^{-1} providing a proportion of particulates of 12.1 - 34.5 %. Similarly, EMDP reported a proportion of 26.2 - 32.1 %, although it should be noted that these values are based on two samples only. Concentrations of codeine, norcodeine, morphine, normorphine, tramadol and dihydrocodeine were detected on SPM, with the proportion < 5.6 % in all samples for each compound.

The concentration of studied antidepressants on SPM was relatively high. The highest concentration determined in this study was for amitriptyline, which ranged from 118.3 – 629.9 ng g^{-1} and resulted in a proportion on solids of 9.4 – 50.3 %. The highest partitioning to solids was determined for fluoxetine, 39.2 – 73.9 %, and norfluoxetine, 36.7 - 89.4 %. Dosulepin reported a proportion on solids ranging from 17.4 – 64.7 %, whilst in contrast, venlafaxine was determined at 0.6 - 3.9 %.

4.2 Data Evaluation

One of the goals of this study was to assess whether or not the adsorption of target drug residues to SPM was at sufficient levels to obstruct the reliable estimation of drug usage through the analysis of the aqueous part of the WWTP influent sample only. The proportion of sorption to SPM is shown in Figure 1. The maximum proportion of analyte sorbed to SPM was < 2.8 % (thus likely to be considered negligible) for cocaine, benzoylecgonine, cocaethylene, MDMA, temazepam and ketamine. Furthermore sorption was < 4.7 % for norcodeine, normorphine, dihydrocodeine, tramadol, oxazepam and nortriptyline. Therefore, publications in which these compounds have been monitored will not be adversely affected with regards to the lack of SPM analysis.

In contrast, the proportion of analyte on SPM could be a cause for concern in relation to some target analytes. Morphine was determined up to a maximum of 5.6 % on SPM, codeine up to 5.2 % and amphetamine up to 8.6 %. Although these values are still relatively low, ideally SPM analysis would be carried out to prevent under-reporting.

The proportion of analyte on SPM for methadone was in the range 8.1 - 18.6 % (mean = 11.5 %), and EDDP, 12.1 – 34.5 % (mean = 18.5 %). The high percentage of analyte on SPM necessitates the analysis of SPM. EDDP was used as an indicator for methadone usage by van Nuijs et al. [9], with the authors reporting extremely low average consumption of methadone (2 mg/day per 1000 inhabitants). The same publication also reports that the use of methadone is uncommon in Europe based on official EMCDDA statistics. It is not clear in this publication whether or not the levels of methadone determined from EDDP were lower than expected in comparison to official statistics, as use was considered negligible in the country where the study was performed (Belgium). However, based on the findings of this study, methadone could have been under-reported by wastewater analysis alone by as much as 35 %. Methadone was employed as the consumption indicator of methadone itself in the work by Postigo et al. [8]. Therefore, based on the results of this study, levels could have possibly been under-reported by up to nearly 20 %.

The partitioning of analytes onto SPM was most significant for antidepressant compounds, which is not surprising given their non-polar nature (see Table S2). The proportion of analyte on SPM was determined at a maximum for dosulepin 64.7 %, amitriptyline 50.3 %, nortriptyline 39.7 %, fluoxetine 73.9 % and norfluoxetine 89.4 %. Somewhat lower than the other antidepressants, venlafaxine was determined at a maximum proportion on solids of 3.9 %, which is likely to be a result of the analyte's more polar nature. High levels of fluoxetine were determined in bio solids collected from a WWTP by Kinney et al. [30], ranging from 100 to 4700 ng $g^{-1}_{\text{organic carbon}}$ and by Radjenovic et al. [21] ranging from 71.9 – 122.7 ng g^{-1}.

5 CONCLUSIONS

This study reports the first PLE methodology for the extraction of drugs of abuse on suspended particulate matter in wastewater. The PLE-SPE-LC-MS/MS procedure developed allows the simultaneous quantification of a wide range of drugs of abuse from complex environmental matrices. Suspended particulate matter was analysed from the UK providing the first comprehensive report of drugs of abuse on SPM.

The developed PLE protocol was optimised through the evaluation of key parameters, including solvent, temperature and number of extraction cycles. A previously developed SPE procedure was used to concentrate PLE extracts, which was further evaluated to assess the impact of higher sample methanol content. This in turn allowed a lower PLE extract dilution volume to be utilised in comparison to other published manuscripts, which recommend a methanol content < 5 %. The methodology was validated through assessment of linearity and range, recovery, accuracy, reproducibility, repeatability, matrix effects, and sensitivity. PLE-SPE-LC-MS/MS recoveries were ≥ 60 % for the majority of compounds when extracted from SPM. Excellent quantification levels were provided for nearly all compounds (54 in total) at < 2.6 ng g^{-1}.

The application of the method to wastewater samples allowed for quantification of 34 compounds on SPM in the range 0.1 (benzoylecgonine) – 629 (amitriptyline) ng g^{-1}. For the majority of compounds this constituted a proportion on SPM < 5 %, although certain compounds reported significant levels. The average proportion on SPM was > 10 % with regards to methadone, EDDP, EMDP, BZP, fentanyl, nortramadol, norpropoxyphene, sildenafil and all antidepressants (dosulepin, amitriptyline, nortriptyline, fluoxetine and norfluoxetine).

ACKNOWLEDGMENTS

David Baker would like to acknowledge the University of Huddersfield Research Fund for funding his PhD studies. The authors would like to thank Yorkshire Water and Dr Ilyas Dawood for assistance. We also greatly acknowledge the advice of Dr Leon Barron during method development.

SUPPLEMENTARY MATERIAL

Supplementary material includes: structures, molecular weights and *CAS* numbers of selected analytes; optimised MRM conditions for the analysis of drugs of abuse by LC-MS/MS and method development parameters for ASE-SPE-LC-MS/MS.

Figure S1 – Negative (a) and positive (b) effect on recovery with an increase in PLE extraction temperature
Figure S2 – Cumulative absolute recovery (%) obtained with successive PLE cycles
Figure S3 - UPLC-MS/MS chromatograms of suspended particulate matter spiked with a concentration of 50 ng g-1 of each compound before PLE
Table S1 – Main parameters reported in analytical methods for the extraction drugs of abuse and pharmaceuticals in environmental solids
Table S2 - Selected analytes and their properties
Table S3 - Optimized MRM conditions and ion ratios
Table S4 – SPE recovery with an increasing methanol to water sample composition
Table S5 – Absolute PLE recoveries with different solvents (uppermost recovery highlighted)
Table S6 – Absolute PLE recoveries at different temperatures
Table S7 – standard deviation values in relation to mean values reported in table 3

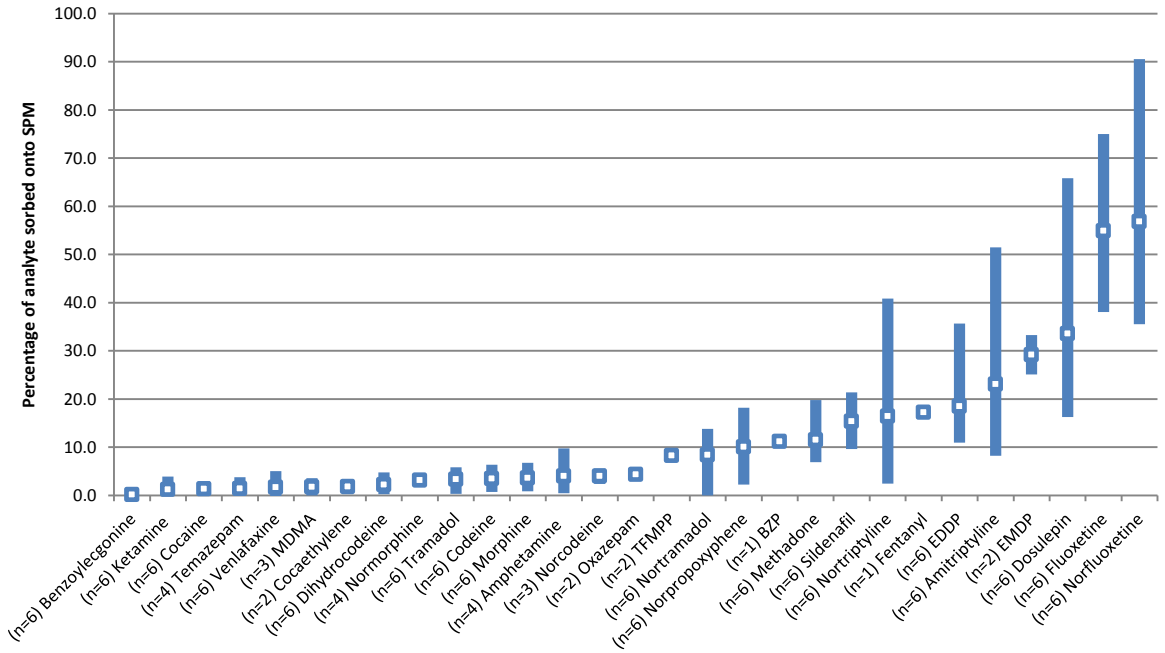
REFERENCES

[1] M.R. Boleda, M.T. Galceran, F. Ventura, Water Res. 43 (2009) 1126.
[2] J. Bones, K.V. Thomas, B. Paull, J. Environ. Monit. 9 (2007) 701.
[3] S. Castiglioni, E. Zuccato, E. Crisci, C. Chiabrando, R. Fanelli, R. Bagnati, Anal. Chem. 78 (2006) 8421.

[4] A.C. Chiaia, C. Banta-Green, J. Field, Environ. Sci. Technol. 42 (2008) 8841.
[5] I. Gonzalez-Marino, J.B. Quintana, I. Rodriguez, R. Cela, J. Chromatogr. A 1217 (2010) 1748.
[6] C. Metcalfe, K. Tindale, H. Li, A. Rodayan, V. Yargeau, Environ. Pollut. 158 (2010) 3179.
[7] B. Kasprzyk-Hordern, V.V.R. Kondakal, D.R. Baker, J. Chromatogr. A 1217 (2010) 4575.
[8] C. Postigo, M.J.L. de Alda, D. Barcelo, Environ. Int. 36 (2010) 75.
[9] A.L.N. van Nuijs, J.-F. Mougel, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Environ. Int. 37 (2011) 612.
[10] P. Vazquez-Roig, V. Andreu, C. Blasco, Y. Pico, Anal. Bioanal. Chem. 397 (2010) 2851.
[11] A. Nieto, F. Borrull, E. Pocurull, R.M. Marcé, TrAC Trends Anal. Chem. 29 (2010) 752.
[12] M.S. Díaz-Cruz, M.J. García-Galán, P. Guerra, A. Jelic, C. Postigo, E. Eljarrat, M. Farré, M.J. López de Alda, M. Petrovic, D. Barceló, TrAC Trends Anal. Chem. 28 (2009) 1263.
[13] A. Kaleta, M. Ferdig, W. Buchberger, J. Sep. Sci. 29 (2006) 1662.
[14] S.A. Smyth, L. Lishman, M. Alaee, S. Kleywegt, L. Svoboda, J.-J. Yang, H.-B. Lee, P. Seto, Chemosphere 67 (2007) 267.
[15] M.I.H. Helaleh, A. Al-Omair, N. Ahmed, B. Gevao, Anal. Bioanal. Chem. 382 (2005) 1127.
[16] K. Stein, M. Ramil, G. Fink, M. Sander, T.A. Ternes, Environ. Sci. Technol. 42 (2008) 6415.
[17] C. Miège, J. Dugay, M.C. Hennion, J. Chromatogr. A 995 (2003) 87.
[18] A. Nieto, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 30 (2007) 979.
[19] A. Nieto, F. Borrull, E. Pocurull, R.M. Marcé, J. Chromatogr. A 1213 (2008) 224.
[20] L. Barron, J. Tobin, B. Paull, J. Environ. Monit. 10 (2008) 353.
[21] J. Radjenović, A. Jelić, M. Petrović, D. Barceló, Anal. Bioanal. Chem. 393 (2009) 1685.
[22] P. Vazquez-Roig, R. Segarra, C. Blasco, V. Andreu, Y. Picó, J. Chromatogr. A 1217 (2010) 2471.
[23] H. Runnqvist, S.A. Bak, M. Hansen, B. Styrishave, B. Halling-Sørensen, E. Björklund, J. Chromatogr. A 1217 (2010) 2447.
[24] D.R. Baker, B. Kasprzyk-Hordern, J. Chromatogr. A 1218 (2011) 1620.
[25] European Commission Decision 2002/657/EC: implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Available at http://ec.europa.eu/food/food/chemicalsafety/residues/lab_analysis_en.htm
[26] ACD/I-lab, (version 12.0) accessed via ACD/chemsketch, Advanced chemistry development Inc. Toronto, ON, Canada. www.acdlabs.com,.
[27] A. Jelic, M. Petrovic, D. Barceló, Talanta 80 (2009) 363.
[28] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Ternes, W. Giger, Anal. Chem. 73 (2001) 3632.
[29] M. Lillenberg, S. Yurchenko, K. Kipper, K. Herodes, V. Pihl, K. Sepp, R. Lõhmus, L. Nei, J. Chromatogr. A 1216 (2009) 5949.
[30] C.A. Kinney, E.T. Furlong, S.D. Zaugg, M.R. Burkhardt, S.L. Werner, J.D. Cahill, G.R. Jorgensen, Environ. Sci. Technol. 40 (2006) 7207.

FIGURES

Figure 1. Experimentally determined proportion of analyte sorbed onto SPM collected from wastewater influent in the UK. White dots represent the mean value, with blue lines representing the range. The number of samples for which the analyte was quantified is shown in brackets next to each compound.



TABLES

Table 1. Performance data for studied compounds extracted by PLE-SPE-LC-MS/MS

Compound	Soil										Suspended particulate matter			Internal standard
	t_R (min)	R^2	Linearity range (ng g^{-1})	Repeat. (% RSD) (n = 5)	Reprod. (% RSD) (n = 3)	Accur. (%) (n = 3)	MDL_{calc} (ng g^{-1})	MQL_{calc} (ng g^{-1})	$MQL_{S/N}$ (ng g^{-1})	MS matrix effect (%) (n = 3)	MDL_{calc} (ng g^{-1})	MQL_{calc} (ng g^{-1})	MS matrix effect (%) (n = 2)	
Stimulants														
Cocaine	11.1	0.999	0.5 - 500	6	4	-6	0.02	0.07	0.05	9 ± 6	0.02	0.08	20 ± 2	Cocaine-D3
Benzoylecgonine	10.6	0.998	0.5 - 500	2	5	6	0.02	0.07	0.03	9 ± 5	0.02	0.10	13 ± 1	Benzoylecgonine-D8
Norbenzoylecgonine	11.2	0.999	0.5 - 500	3	3	2	0.02	0.08	0.02	15 ± 4	0.02	0.09	24 ± 1	Benzoylecgonine-D8
Norcocaine	12.1	0.999	0.5 - 500	4	10	5	0.01	0.06	0.03	-13 ± 10	0.02	0.06	-7 ± 1	Cocaine-D3
Cocaethylene	13.2	0.999	0.5 - 500	5	4	4	0.02	0.09	0.01	24 ± 2	0.02	0.09	34 ± 1	Cocaethylene-D8
Anhydroecgonine M. E.	3.3	0.995	0.5 - 100	7	10	3	0.16	0.78	0.53	24 ± 4	1.87	9.33	73 ± 1	Cocaine-D3
Ecgonidine	2.9	0.996	5.0 - 200	17	7	7	0.77	3.84	2.44	42 ± 3	4.07	20.33	70 ± 2	Benzoylecgonine-D8
Amphetamine	6.2	0.999	0.5 - 500	4	6	-8	0.05	0.25	0.17	-20 ± 19	0.05	0.23	-118 ± 10	Amphetamine-D11
Methamphetamine	6.8	0.999	0.5 - 500	3	1	-11	0.02	0.06	0.05	-4 ± 12	0.02	0.08	-17 ± 1	Methamphetamine-D14
Methcathinone	4.6	0.999	0.5 - 500	7	14	3	0.04	0.25	0.15	-68 ± 24	0.18	1.19	-54 ± 5	Methamphetamine-D14
BZP	3.7	0.994	5.0 - 500	21	42	1	1.31	2.62	0.31	-9 ± 4	1.61	3.23	58 ± 0	PCP-D5
TFMPP	13.7	0.997	0.5 - 200	5	5	7	0.02	0.08	0.07	2 ± 6	0.02	0.09	16 ± 0	PCP-D5
Hallucinogens														
MDA	6.9	0.999	0.5 - 500	3	3	-4	0.04	0.37	0.20	7 ± 9	0.05	0.48	5 ± 3	MDA-D5
MDMA	7.1	0.999	0.5 - 500	2	1	6	0.02	0.08	0.08	5 ± 6	0.03	0.10	21 ± 3	MDMA-D5
MDEA	8.8	0.998	0.5 - 500	2	4	10	0.02	0.08	0.07	7 ± 6	0.02	0.08	17 ± 1	MDEA-D5

MBDB	9.9	0.998	0.5	-	500	4	6	-13	0.02	0.08	0.03	14 ± 5	0.02	0.07	10 ± 1	MBDB-D5
BDB	10.0	0.992	0.5	-	150	7	12	-18	0.06	0.39	0.17	10 ± 8	0.04	0.25	-41 ± 2	MBDB-D5
Mescaline	5.7	0.999	5.0	-	500	3	2	-5	0.29	0.59	0.76	-9 ± 12	2.87	5.74	85 ± 1	Mescaline-D9
LSD	13.1	0.998	0.5	-	500	4	6	5	0.03	0.12	0.05	18 ± 2	0.02	0.09	27 ± 0	LSD-D3
O-H-LSD	8.6	0.999	0.5	-	500	4	10	5	0.03	0.10	0.02	14 ± 5	0.03	0.13	31 ± 0	LSD-D3
Opioids and morphine derivatives																
Heroin	10.8	0.999	0.5	-	500	4	3	-5	0.07	0.47	0.10	7 ± 3	0.09	0.60	26 ± 2	Heroin-D9
6-acetylmorphine	5.3	0.999	0.5	-	500	9	9	-3	0.04	0.29	0.09	-7 ± 7	0.07	0.50	29 ± 2	Codeine-D6
Codeine	4.0	0.999	0.5	-	500	3	2	1	0.05	0.30	0.16	2 ± 7	0.17	1.15	69 ± 0	Codeine-D6
Norcodeine	4.1	0.998	0.5	-	500	4	1	-12	0.06	0.29	0.28	-6 ± 9	0.15	0.73	44 ± 0	Codeine-D6
Oxycodone	4.5	0.998	0.5	-	500	4	3	3	0.05	0.35	0.12	1 ± 6	0.15	0.97	36 ± 1	Oxycodone-D6
Oxymorphone	3.3	0.997	0.5	-	200	4	2	8	0.05	0.35	0.33	-8 ± 7	0.32	2.10	29 ± 0	Oxycodone-D6
Morphine	3.2	0.998	0.5	-	250	8	4	4	0.07	0.36	0.22	-2 ± 9	0.35	1.73	62 ± 5	Morphine-D6
Normorphine	3.2	0.997	0.5	-	250	6	2	7	0.09	0.46	0.45	12 ± 5	0.51	2.53	69 ± 3	Morphine-D6
Dihydrocodeine	3.9	0.998	0.5	-	500	3	3	4	0.05	0.32	0.06	-1 ± 2	0.15	1.02	45 ± 1	Dihydrocodeine-D6
Buprenorphine	16.4	0.997	0.5	-	250	7	2	8	0.15	0.73	0.19	27 ± 2	0.17	0.84	61 ± 2	Buprenorphine-D4
Norbuprenorphine	14.4	0.996	0.5	-	250	8	7	12	0.12	0.60	0.17	21 ± 1	0.12	0.59	42 ± 1	Buprenorphine-D4
Methadone	19.1	0.999	0.5	-	500	9	8	3	0.02	0.07	0.02	4 ± 7	0.03	0.11	49 ± 3	Methadone-D9
EDDP	15.7	0.997	0.5	-	500	10	6	-15	0.05	0.20	0.06	26 ± 2	0.04	0.18	62 ± 7	EDDP-D3
EMDP	20.3	0.997	0.5	-	500	12	3	3	0.03	0.11	0.04	4 ± 4	0.03	0.10	21 ± 3	Methadone-D9
Fentanyl	14.5	0.979	0.5	-	150	31	15	19	0.06	0.24	0.04	22 ± 2	0.03	0.10	36 ± 2	Fentanyl-D5
Norfentanyl	10.4	0.996	0.5	-	500	5	4	9	0.02	0.08	0.02	12 ± 6	0.03	0.12	23 ± 1	Dihydrocodeine-D6
Propoxyphene	18.6	0.999	0.5	-	500	4	5	-4	0.03	0.34	0.10	-87 ± 25	0.02	0.25	-191 ± 3	Propoxyphene-D11

Norpropoxyphene	19.0	0.997	5.0	-	500	7	7	-15	0.49	2.45	1.12	-66	±	19	0.38	1.90	-91	± 21	Norpropoxyphene-D5
Tramadol	10.7	0.998	0.5	-	500	2	2	11	0.03	0.34	0.04	11	±	3	0.06	0.62	47	± 1	Codeine-D6
Nortramadol	11.7	0.997	5.0	-	500	6	19	-8	0.30	1.51	3.83	-118	±	25	0.18	0.92	-300	± 22	Codeine-D6
Benzodiazepines																			
Temazepam	22.2	0.997	0.5	-	500	5	2	-4	0.10	0.41	0.29	3	±	2	0.21	0.86	-1	± 2	Temazepam-D5
Diazepam	23.1	0.999	0.5	-	500	1	6	4	0.06	0.43	0.02	24	±	1	0.44	2.93	87	± 1	Diazepam-D5
Nordiazepam	22.8	0.999	0.5	-	500	3	6	-5	0.06	0.39	0.02	4	±	5	2.44	16.26	97	± 0	Diazepam-D5
Nitrazepam	19.9	0.996	0.5	-	500	5	16	-2	0.07	0.44	0.10	-8	±	6	0.09	0.58	3	± 4	Diazepam-D5
7-aminonitrazepam	5.7	0.996	5.0	-	500	8	12	-13	0.23	1.52	0.24	-13	±	5	0.11	0.71	-4	± 6	EDDP-D3
Oxazepam	21.8	0.998	5.0	-	500	1	4	3	0.15	0.98	0.16	14	±	2	0.39	2.58	31	± 1	Oxazepam-D5
Chlordiazepoxide	16.9	0.998	0.5	-	500	5	11	-11	0.05	0.33	0.10	-6	±	7	0.04	0.26	-26	± 2	Diazepam-D5
Antidepressants																			
Dosulepin	18.2	0.997	0.5	-	500	6	6	-5	0.09	0.62	0.09	8	±	7	0.14	0.90	70	± 14	LSD-D3
Amitriptyline	19.5	0.996	0.5	-	250	9	14	-4	0.08	0.55	0.22	2	±	6	0.06	0.40	40	± 18	LSD-D3
Nortriptyline	19.9	0.996	5.0	-	250	8	8	-3	0.11	0.76	0.09	18	±	5	0.11	0.74	54	± 2	LSD-D3
Fluoxetine	20.3	0.997	5.0	-	250	37	48	2	0.10	0.68	1.84	-10	±	6	0.07	0.47	29	± 0	Fluoxetine-D6
Norfluoxetine	20.5	0.997	5.0	-	500	35	52	-11	0.09	0.58	0.17	-47	±	8	0.03	0.19	-165	± 15	Fluoxetine-D6
Venlafaxine	14.6	0.998	0.5	-	500	8	17	4	0.06	0.40	0.10	6	±	11	0.06	0.38	24	± 0	LSD-D3
Dissociative anaesthetics																			
Phencyclidine	13.8	0.996	0.5	-	250	5	5	10	0.02	0.06	0.09	-53	±	23	0.01	0.05	-88	± 2	PCP-D5
Ketamine	9.9	0.999	0.5	-	500	1	2	5	0.02	0.08	0.06	22	±	2	0.03	0.12	40	± 0	Ketamine-D4

Norketamine	9.9	0.998	0.5	-	500	2	2	3	0.07	0.44	0.03	18	±	2	0.11	0.75	34	± 1	Ketamine-D4
Other																			
Methaqualone	20.2	0.996	0.5	-	200	5	7	-11	0.07	0.49	0.03	29	±	7	0.05	0.35	13	± 4	Methaqualone-D7
Sildenafil	17.8	0.996	5.0	-	500	7	10	-3	0.39	0.78	0.19	-11	±	4	0.27	0.54	-2	± 3	PCP-D5
Drug precursors																			
Ephedrine	4.9	0.998	0.5	-	500	3	2	13	0.27	0.54	0.20	-33	±	16	0.32	0.64	-123	± 3	Amphetamine-D11
Norephedrine	4.0	0.997	5.0	-	500	4	14	0	0.20	0.99	0.49	-212	±	47	0.47	2.36	-363	± 7	Amphetamine-D11

Table 2. Absolute SPE, PLE and PLE-SPE-LC-MS/MS recovery, and relative recovery for all analytes

Compound	Absolute and relative recovery (50 ng g^{-1})							
	Soil				Suspended particulate matter			
	PLE absolute rec. (%)[a]	SPE absolute rec. (%)[b]	PLE-SPE-LC-MS/MS absolute rec. (%)[c]	PLE-SPE-LC-MS/MS Relative rec. (%)[d]	PLE absolute rec. (%)[a]	SPE absolute rec. (%)[b]	PLE-SPE-LC-MS/MS absolute rec. (%)[c]	PLE-SPE-LC-MS/MS Relative rec.(%)[d]
Stimulants								
Cocaine	87 ± 8	90 ± 8	78 ± 7	94 ± 6	88 ± 4	92 ± 4	81 ± 4	93 ± 7
Benzoylecgonine	91 ± 3	88 ± 9	80 ± 3	95 ± 2	91 ± 5	66 ± 5	60 ± 3	97 ± 8
Norbenzoylecgonine	91 ± 3	80 ± 7	73 ± 2	87 ± 2	87 ± 2	80 ± 1	70 ± 1	113 ± 17
Norcocaine	91 ± 6	85 ± 5	77 ± 5	93 ± 3	86 ± 3	89 ± 2	77 ± 3	89 ± 6
Cocaethylene	88 ± 6	88 ± 6	77 ± 5	92 ± 5	91 ± 4	92 ± 1	84 ± 3	89 ± 6
Anhydroecgonine M. E.	86 ± 4	49 ± 8	42 ± 2	51 ± 4	68 ± 6	15 ± 0	10 ± 1	11 ± 1
Ecgonidine	72 ± 13	16 ± 1	11 ± 2	13 ± 3	77 ± 14	5 ± 0	4 ± 1	7 ± 0
Amphetamine	90 ± 8	93 ± 9	84 ± 8	109 ± 3	74 ± 2	69 ± 8	51 ± 1	94 ± 10
Methamphetamine	90 ± 9	84 ± 7	76 ± 7	109 ± 7	78 ± 5	73 ± 12	57 ± 3	85 ± 9
Methcathinone	88 ± 8	69 ± 9	61 ± 5	87 ± 8	50 ± 2	28 ± 6	14 ± 1	20 ± 0
BZP	35 ± 5	50 ± 4	17 ± 3	32 ± 4	56 ± 11	67 ± 4	37 ± 7	55 ± 9
TFMPP	90 ± 13	68 ± 9	61 ± 9	115 ± 17	81 ± 4	79 ± 5	64 ± 3	94 ± 7
Hallucinogens								
MDA	88 ± 7	84 ± 9	73 ± 6	101 ± 7	77 ± 1	71 ± 15	55 ± 1	82 ± 6
MDMA	88 ± 6	76 ± 5	67 ± 5	114 ± 3	81 ± 4	78 ± 9	63 ± 3	95 ± 6
MDEA	92 ± 5	77 ± 6	71 ± 4	106 ± 3	87 ± 5	83 ± 9	72 ± 4	95 ± 5

MBDB	90	±	3	80	±	6	72	±	3	96	±	2	88	±	5	85	±	1	74	±	5	92	±	8
BDB	86	±	7	83	±	8	72	±	5	95	±	5	81	±	5	89	±	8	72	±	5	88	±	8
Mescaline	92	±	9	85	±	7	78	±	8	93	±	6	58	±	5	103	±	35	60	±	5	91	±	1
LSD	81	±	10	64	±	7	52	±	6	104	±	11	86	±	4	89	±	4	76	±	4	90	±	8
O-H-LSD	88	±	6	66	±	5	58	±	4	116	±	8	83	±	6	66	±	0	55	±	4	65	±	3
Opiods and morphine derivatives																								
Heroin	85	±	7	68	±	7	58	±	5	93	±	4	84	±	2	67	±	2	56	±	2	89	±	7
6-acetylmorphine	77	±	9	104	±	7	80	±	10	94	±	9	90	±	4	78	±	5	70	±	3	106	±	21
Codeine	89	±	6	95	±	8	85	±	5	100	±	5	91	±	5	77	±	2	70	±	4	105	±	19
Norcodeine	90	±	6	90	±	5	81	±	6	95	±	4	83	±	2	73	±	4	61	±	2	92	±	20
Oxycodone	84	±	7	86	±	10	72	±	6	91	±	5	78	±	0	52	±	3	40	±	0	91	±	6
Oxymorphone	89	±	8	75	±	12	67	±	6	84	±	7	56	±	6	30	±	3	17	±	2	38	±	1
Morphine	72	±	10	94	±	8	67	±	9	99	±	15	78	±	13	49	±	5	38	±	7	122	±	7
Normorphine	67	±	8	92	±	11	61	±	8	90	±	13	75	±	13	43	±	6	32	±	6	103	±	6
Dihydrocodeine	88	±	6	89	±	8	78	±	6	97	±	3	83	±	10	53	±	5	44	±	5	113	±	9
Buprenorphine	69	±	9	69	±	12	47	±	6	114	±	17	84	±	2	91	±	0	76	±	2	91	±	10
Norbuprenorphine	69	±	9	76	±	11	53	±	7	127	±	20	84	±	3	87	±	0	73	±	2	88	±	11
Methadone	107	±	17	71	±	10	76	±	12	113	±	18	96	±	4	94	±	9	91	±	3	115	±	7
EDDP	87	±	18	40	±	5	34	±	7	97	±	12	91	±	11	83	±	9	75	±	9	142	±	14
EMDP	84	±	12	55	±	14	47	±	7	69	±	10	76	±	4	82	±	1	62	±	3	79	±	5
Fentanyl	38	±	6	70	±	7	27	±	4	110	±	33	89	±	5	87	±	3	78	±	4	94	±	4
Norfentanyl	83	±	7	87	±	9	72	±	6	89	±	5	79	±	8	69	±	3	55	±	6	140	±	13
Propoxyphene	97	±	9	80	±	9	78	±	7	103	±	8	84	±	1	82	±	5	69	±	1	90	±	8

Norpropoxyphene	109	±	15	56	±	6	61	±	8	145	±	23	76	±	7	91	±	3	69	±	6	94	±	6
Tramadol	92	±	3	89	±	6	82	±	3	97	±	2	80	±	16	95	±	4	76	±	15	112	±	6
Nortramadol	91	±	8	84	±	8	76	±	7	90	±	9	72	±	2	94	±	28	68	±	2	103	±	22
Benzodiazepines																								
Temazepam	91	±	5	14	±	1	12	±	1	101	±	5	86	±	7	7	±	1	6	±	0	72	±	10
Diazepam	91	±	3	84	±	5	77	±	2	97	±	2	81	±	2	81	±	7	66	±	1	94	±	10
Nordiazepam	81	±	4	82	±	6	67	±	4	85	±	4	74	±	10	78	±	8	58	±	8	83	±	21
Nitrazepam	81	±	4	64	±	8	52	±	2	66	±	3	84	±	4	53	±	4	44	±	2	63	±	5
7-aminonitrazepam	54	±	8	27	±	6	15	±	2	41	±	5	75	±	0	45	±	3	34	±	0	64	±	2
Oxazepam	85	±	5	35	±	4	30	±	2	94	±	2	80	±	9	18	±	3	14	±	2	64	±	1
Chlordiazepoxide	83	±	5	86	±	8	72	±	4	91	±	5	87	±	3	89	±	3	77	±	3	110	±	17
Antidepressants																								
Dosulepin	108	±	7	51	±	9	44	±	25	109	±	4	108	±	1	87	±	10	93	±	1	110	±	5
Amitriptyline	123	±	11	47	±	9	46	±	26	115	±	7	108	±	2	98	±	16	106	±	2	125	±	6
Nortriptyline	127	±	14	39	±	8	40	±	23	100	±	8	119	±	8	62	±	1	74	±	5	87	±	3
Fluoxetine	123	±	17	34	±	7	34	±	19	127	±	13	106	±	15	71	±	9	75	±	11	115	±	20
Norfluoxetine	130	±	21	28	±	7	29	±	17	110	±	14	91	±	17	54	±	9	50	±	9	75	±	10
Venlafaxine	75	±	9	89	±	9	67	±	8	133	±	13	91	±	3	95	±	2	86	±	3	102	±	7
Dissociative anaesthetics																								
Phencyclidine	62	±	10	86	±	8	53	±	8	99	±	12	75	±	5	78	±	10	58	±	4	86	±	8
Ketamine	90	±	4	87	±	7	78	±	4	95	±	3	92	±	0	76	±	4	70	±	0	94	±	6
Norketamine	91	±	5	75	±	6	68	±	4	83	±	3	85	±	2	60	±	6	51	±	1	68	±	3

Other																						
Methaqualone	96	±	7	75	±	11	72	±	5	89	±	5	92	±	4	89	±	2	81	±	3	94	±	8
Sildenafil	94	±	16	61	±	14	58	±	10	115	±	18	98	±	0	92	±	5	90	±	0	106	±	4
Drug precursors																						
Ephedrine	92	±	7	76	±	10	70	±	5	92	±	5	66	±	1	53	±	2	35	±	1	64	±	7
Norephedrine	92	±	7	89	±	18	81	±	6	106	±	5	56	±	8	41	±	1	23	±	3	42	±	0

[a] Absolute recovery for PLE; Soil (n = 5)/SPM (n = 2) spiked before PLE and compared to soil/SPM sample spiked after PLE (before SPE)

[b] Absolute recovery for SPE (including evaporation and reconstitution); Soil (n = 3)/SPM (n = 2) spiked after PLE (before SPE) and compared to soil/SPM sample spiked during reconstitution

[c] Absolute recovery for PLE-SPE-LC-MS/MS; Soil (n = 3)/SPM (n = 2) spiked before PLE in comparison to soil sample spiked during reconstitution

[d] Relative to surrogate/internal standard; same samples as described in [c]

Table 3. Concentration of analytes sorbed onto suspended particulate matter (ng g^{-1}), dissolved in wastewater (ng L^{-1}) and the percentage of the total concentration determined on suspended particulate matter. Standard deviations in relation to the mean values shown in this table are listed in table S7.

Compound	**October**									**November**								
	WWTP A			**WWTP B**			**WWTP C**			**WWTP A**			**WWTP B**			**WWTP C**		
	0.34 mg SPM per Litre			0.31 mg SPM per Litre			0.40 mg SPM per Litre			0.29 mg SPM per Litre			0.36 mg SPM per Litre			0.31 mg SPM per Litre		
	SPM[a]	**WW[b]**	**%[c]**	**SPM[a]**	**WW[b]**	**%[c]**	**SPM[a]**	**WW[b]**	**%[c]**	**SPM[a]**	**WW[b]**	**%[c]**	**SPM[a]**	**WW[b]**	**%[c]**	**SPM[a]**	**WW[b]**	**%[c]**
Stimulants																		
Cocaine	1.8	43.2	**1.4**	2.6	43.9	**1.8**	2.7	81.9	**1.3**	1.8	37.5	**1.3**	2.1	51.5	**1.5**	2.0	66.7	**0.9**
Benzoylecgonine	0.8	192.8	**0.1**	1.1	172.6	**0.2**	0.1	205.1	**0.0**	0.9	140.9	**0.2**	1.0	242.2	**0.2**	0.1	154.9	**0.0**
Norbenzoylecgonine	ND	5.4	-	ND	5.0	-	ND	6.3	-	ND	4.6	-	ND	7.6	-	ND	4.3	-
Norcocaine	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Cocaethylene	<MQL	1.4	-	ND	1.1	-	0.2	5.6	**1.4**	ND	1.3	-	ND	1.8	-	0.3	3.5	**2.2**
Anhydroecgonine M.E.	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Ecgonidine	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Amphetamine[d]	5.2	46.0	**3.7**	13.1	42.7	**8.6**	5.2	124.7	**1.6**	ND	48.3	-	ND	116.4	-	17.0	255.5	**2.0**
Methamphetamine	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	0.6	-
Methcathinone	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
BZP	ND	36.1	-	ND	21.3	-	ND	21.3	-	24.3	55.6	**11.2**	ND	ND	-	ND	ND	-
TFMPP[d]	0.3	<MQL	-	ND	1.3	-	2.0	8.8	**8.1**	ND	ND	-	ND	3.6	-	0.7	2.2	**8.3**
Hallucinogens																		
MDA	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
MDMA	ND	1.8	-	ND	2.3	-	0.7	10.6	**2.4**	0.2	2.8	**1.7**	ND	1.8	-	0.5	12.6	**1.2**
MDEA	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-

MBDB	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
BDB	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Mescaline	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
LSD	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
O-H-LSD	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Opiods and morphine derivatives																		
Heroin	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
6-acetylmorphine	ND	5.9	-	ND	5.9	-	ND	21.6	-	ND	4.5	-	ND	3.6	-	ND	7.3	-
Codeine	98.3	955.7	**3.3**	77.2	949.9	**2.4**	240.0	2041.5	**4.4**	128.1	667.0	**5.2**	59.0	1101.9	**1.9**	130.0	1075.2	**3.6**
Norcodeine	5.7	52.7	**3.5**	ND	50.0	-	10.3	96.7	**4.0**	7.2	45.2	**4.4**	ND	60.3	-	ND	61.7	-
Oxycodone	<MQL	10.2	-	<MQL	6.2	-	<MQL	8.8	-	<MQL	8.0	-	<MQL	24.2	-	<MQL	14.5	-
Oxymorphone	<MQL	<MQL	-	ND	<MQL	-	<MQL	17.4	-	ND	<MQL	-	ND	<MQL	-	ND	ND	-
Morphine	33.2	335.0	**3.2**	22.7	337.0	**2.0**	115.8	777.9	**5.6**	25.3	156.4	**4.4**	18.6	239.7	**2.7**	33.1	274.3	**3.6**
Normorphine	11.9	133.6	**2.9**	ND	122.5	-	15.7	201.0	**3.0**	12.9	106.2	**3.4**	ND	115.2	-	16.4	147.4	**3.3**
Dihydrocodeine	13.3	247.1	**1.8**	13.2	276.3	**1.4**	30.3	320.6	**3.6**	11.9	126.9	**2.6**	10.3	226.5	**1.6**	16.1	239.5	**2.1**
Buprenorphine	1.0	ND	-	ND	ND	-	3.0	ND	-	ND	ND	-	ND	ND	-	1.4	ND	-
Norbuprenorphine	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Methadone	31.5	69.4	**13.2**	23.0	70.0	**9.1**	57.6	99.6	**18.6**	24.3	69.1	**9.2**	19.4	80.1	**8.1**	41.0	104.8	**10.9**
EDDP	61.9	126.3	**14.1**	56.6	122.0	**12.4**	194.0	145.9	**34.5**	30.1	59.7	**12.7**	55.1	144.5	**12.1**	144.7	134.1	**25.2**
EMDP	0.7	ND	-	0.3	<MQL	-	2.3	1.9	**32.1**	0.2	ND	-	0.3	<MQL	-	1.3	1.2	**26.2**
Fentanyl	ND	ND	-	ND	ND	-	0.6	1.1	**17.2**	ND	ND	-	ND	<MQL	-	ND	<MQL	-
Norfentanyl	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Propoxyphene	ND	ND	-	ND	ND	-	ND	<MQL	-	ND	ND	-	ND	ND	-	ND	ND	-

Norpropoxyphene	48.8	203.1	**7.5**	51.6	76.6	**17.0**	133.9	274.7	**16.2**	53.7	280.6	**5.2**	15.2	154.8	**3.4**	59.6	151.8	**10.9**
Tramadol	109.3	1039.0	**3.4**	116.0	1095.8	**3.1**	199.2	1839.2	**4.1**	43.3	841.4	**1.5**	120.4	1327.0	**3.2**	435.2	2758.7	**4.7**
Nortramadol	61.9	335.5	**5.8**	121.6	256.7	**12.6**	206.3	616.3	**11.7**	9.8	248.5	**1.1**	196.1	830.7	**7.9**	383.8	946.1	**11.2**
Benzodiazepines																		
Temazepam	2.2	77.5	**0.9**	4.0	100.7	**1.2**	ND	64.2	**-**	5.9	63.8	**2.6**	2.1	81.4	**0.9**	ND	43.8	**-**
Diazepam	ND	<MQL	**-**	ND	<MQL	**-**	ND	<MQL	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**
Nordiazepam	ND	26.8	**-**	ND	16.1	**-**	ND	12.3	**-**	ND	17.8	**-**	ND	36.8	**-**	ND	17.6	**-**
Nitrazepam	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**
7-aminonitrazepam	2.6	ND	**-**	ND	ND	**-**	1.0	ND	**-**	1.3	ND	**-**	ND	ND	**-**	1.6	ND	**-**
Oxazepam	<MQL	18.7	**-**	ND	23.0	**-**	5.9	52.2	**4.3**	<MQL	12.4	**-**	ND	18.8	**-**	4.2	27.8	**4.5**
Chlordiazepoxide	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**
Antidepressants																		
Dosulepin	174.2	90.7	**39.2**	73.0	87.9	**20.2**	299.1	64.7	**64.7**	128.7	155.2	**19.3**	81.1	138.7	**17.4**	179.9	81.8	**40.7**
Amitriptyline	325.2	341.7	**24.2**	118.3	347.2	**9.4**	476.2	186.2	**50.3**	180.2	201.9	**20.4**	125.9	295.8	**13.3**	629.2	741.1	**20.9**
Nortriptyline	18.4	25.0	**19.8**	3.7	30.7	**3.6**	37.6	22.6	**39.7**	9.4	38.6	**6.5**	6.9	41.9	**5.6**	30.4	30.9	**23.5**
Fluoxetine	109.6	25.5	**59.1**	77.5	26.8	**46.8**	197.1	27.6	**73.9**	72.1	32.1	**39.2**	89.7	32.7	**49.8**	199.2	40.2	**60.7**
Norfluoxetine	54.6	12.4	**59.6**	37.7	8.6	**57.3**	87.1	4.1	**89.4**	35.0	17.4	**36.7**	53.4	25.2	**43.4**	71.7	18.6	**54.6**
Venlafaxine	4.2	87.9	**1.6**	6.9	97.6	**2.1**	15.3	149.7	**3.9**	2.9	138.6	**0.6**	3.6	134.9	**1.0**	5.9	178.2	**1.0**
Dissociative anaesthetics																		
Phencyclidine	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**	ND	ND	**-**
Ketamine	5.3	62.7	**2.8**	1.5	85.5	**0.5**	3.3	81.9	**1.6**	1.9	45.7	**1.2**	1.0	56.6	**0.6**	7.2	349.4	**0.6**
Norketamine	ND	6.2	**-**	ND	<MQL	**-**	ND	16.2	**-**	ND	<MQL	**-**	ND	5.3	**-**	ND	20.1	**-**

Other																		
Methaqualone	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-
Sildenafil	10.0	18.1	**15.7**	5.7	14.3	**10.8**	14.8	39.1	**13.0**	8.1	9.2	**20.2**	12.1	22.3	**16.4**	10.5	17.2	**16.0**
Drug precursors																		
Ephedrine	ND	800.2	-	ND	1079.5	-	ND	1071.0	-	ND	501.0	-	ND	676.2	-	ND	529.1	-
Norephedrine	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-

[a] Concentration of analyte on suspended particulate matter (ng g^{-1}) (n = 2)

[b] Concentration of analyte in wastewater (ng L^{-1}) (n = 2)

[c] Proportion of analyte on suspended particulate matter (n = 2)

[d] MS ion ratio in all samples was outside the permitted tolerance range; therefore could not be reliably quantified. Nevertheless results are shown for information purposes

Supplementary material

Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry

David R. Baker[1] and Barbara Kasprzyk-Hordern[2*]

[1]University of Huddersfield, Department of Chemical and Biological Sciences, School of Applied Sciences, Queensgate, Huddersfield HD1 3DH, UK

[2] University of Bath, Department of Chemistry, Faculty of Science, Bath BA2 7AY, UK

[*] Corresponding author: E-mail: b.kasprzyk-hordern@bath.ac.uk; Fax: +44(0) 1225 386231; Tel: +44 (0) 1225 385013

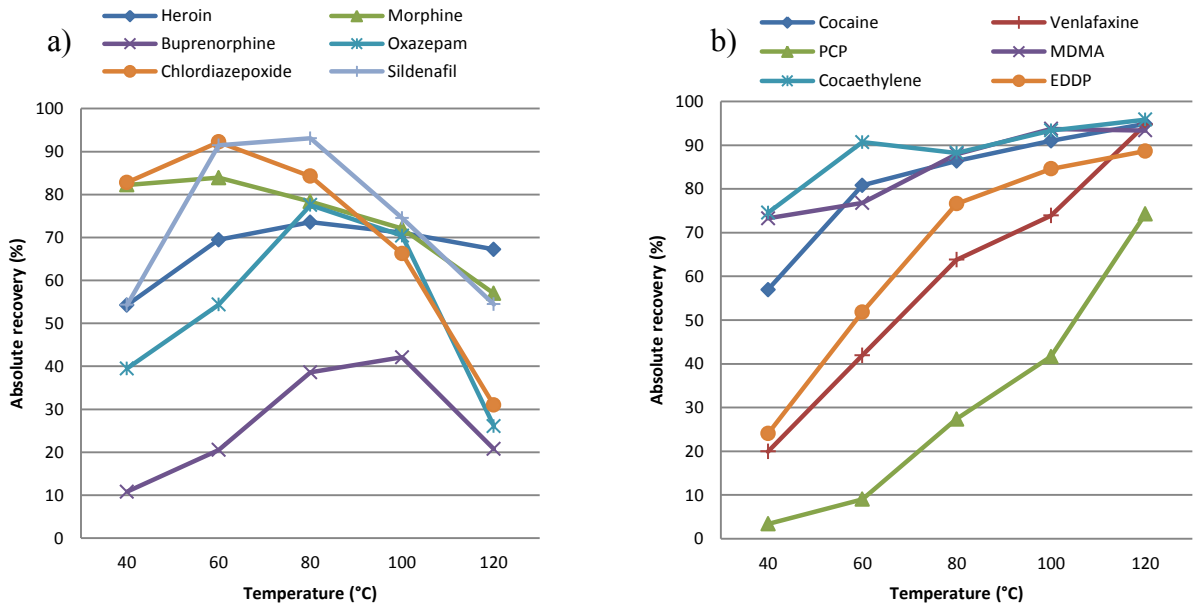


Figure S1 – Negative (a) and positive (b) effect on recovery with an increase in PLE extraction temperature

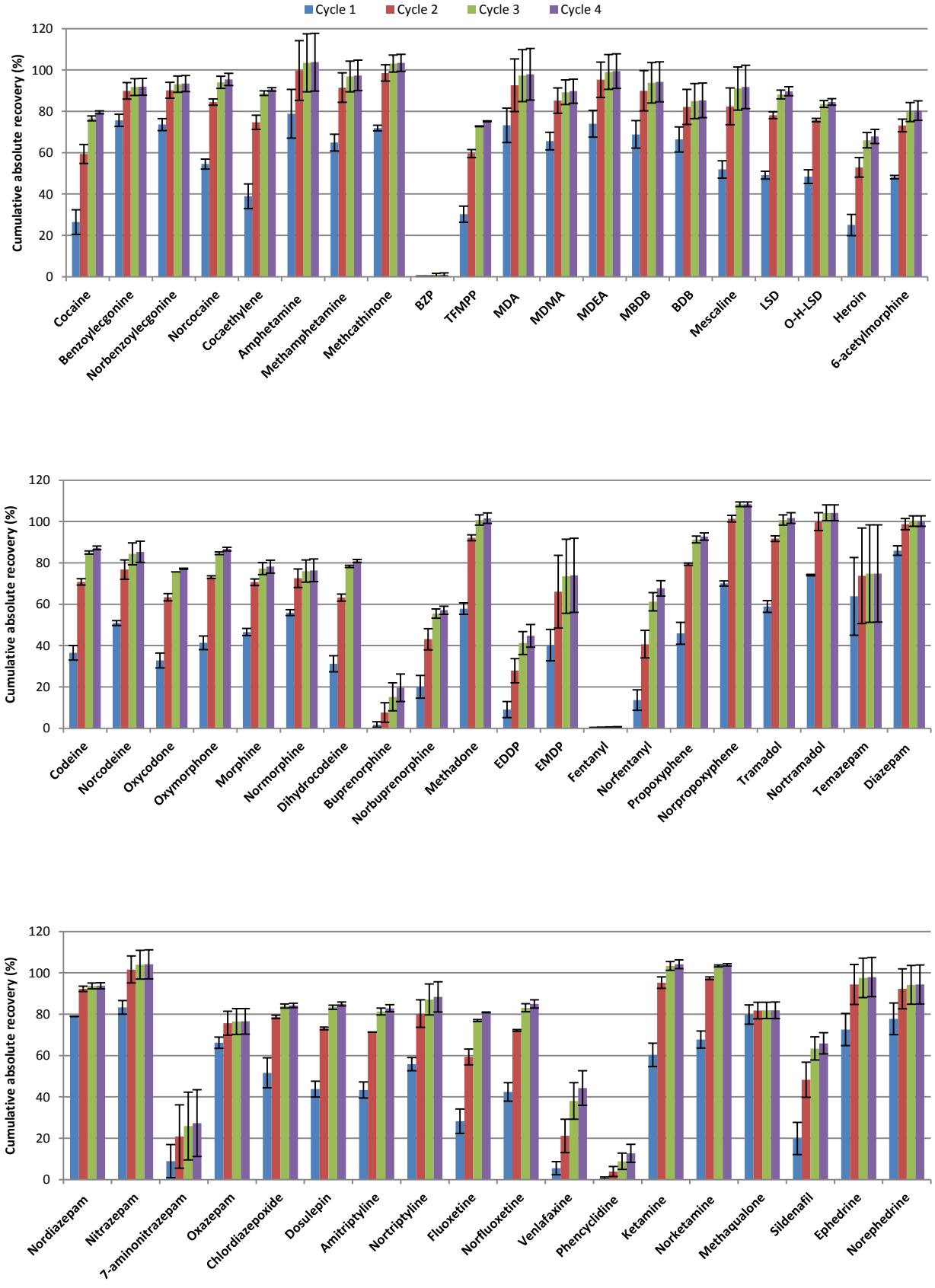


Figure S2 – Cumulative absolute recovery (%) obtained with successive PLE cycles

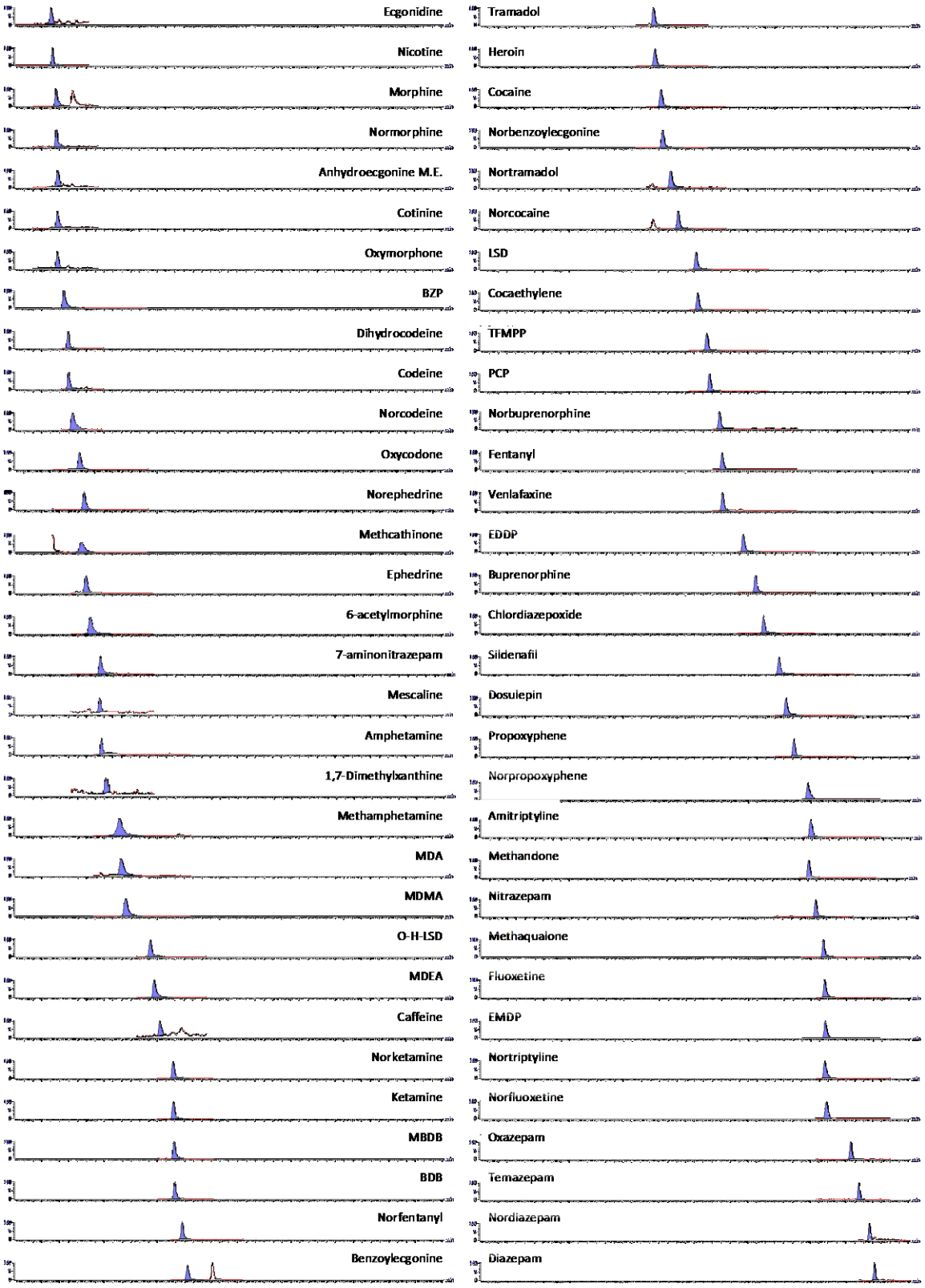


Figure S3 - UPLC-MS/MS chromatograms of suspended particulate matter spiked with a concentration of 50 ng g^{-1} of each compound before PLE

Table S1 – Main parameters reported in analytical methods for the extraction drugs of abuse and pharmaceuticals in environmental solids

No. of analytes	Sample matrix (sample size)	Pre-treatment and storage	PLE conditions					Spiking	Absolute/relative recovery	Post-treatment	Reference
			Cell matrix; Cell size	Solvent	Temp.; Pressure	Cycles x static time	Flush vol.; Purge time				
1[a]	Sewage sludge (1 g)	Centrifugation and Karl-Fischer titration Sludge sonicated in 20 mL of 50 mM formic acid/MeOH (80/20, v/v)	N/A	N/A	N/A	N/A	N/A	NR	NR	Sonicated sample made up to 500 mL with H_2O; SPE - Oasis HLB (500 mg) (pH 10)	Kaleta et al. [1]
10[b]	Sewage sludge (5 g)	Samples stored in the freezer until analysis Sludge lyophilised, pestle and mortared and sieved (< 125 µm)	Aluminium oxide; 33 mL extraction cell	50 mM phosphoric acid/MeOH (50/50, v/v)	100 °C; 1450 psi	2 x 15 min	150 %; 300 sec	Spiked directly onto freeze dried sludge and stirred intensively	Absolute recovery[c]: 70 – 120% (9/10) 68% (1/10)	PLE extract (approx. 40 mL) filtered (0.45 µm microfilter) and analysed by LC	Nieto et al. [2]
27[d]	Sewage sludge (1 g) Soil (2.7 g)	Samples stored at -5 °C until analysis Soil/sludge lyophilised and sieved (<0.7 mm)	Sea sand; 33 mL extraction cell	MeOH/H_2O (50/50, v/v)	60 °C; 1500 psi	2 x 5 min	100 %; 60 sec	Spiking carried out directly onto soil/sludge	Absolute recovery[e,f]: 70 - >130% (16/24) 40 – 65% (7/24) 2% (1/24) Absolute recovery[e]: 70 - >130% (18/27) 50 - 60% (4/27) <30% (4/27)	PLE extract (approx.53 mL) made up to 1 L with H_2O; SPE - Oasis HLB (200 mg) (pH 5.5)	Barron et al. [3]
12[g]	River sediments (1 g)	Samples air dried and sieved (< 2 mm)	Quartz sand; 11 mL extraction cell	MeOH/H_2O (50/50 v/v)	100 °C; 1450 psi	3 x 5 min	120 %; 30 sec	Analytes spiked onto mixture of sediment and sand	Absolute recovery[h]: 71 – 82% (6/12) 43 – 61% (5/12) 32% (1/12) Relative recovery: 96-134% (12/12)	PLE extract (approx. 35 mL) made up to approx. 615 mL with ground water; SPE - Oasis HLB (200 mg) (pH 7)	Stein et al. [4]
31[i]	Sewage sludge (1 g)	Centrifugation and lyophilisation Stored at -40 °C until analysis	Hydromatrix; 11 mL extraction cell	MeOH/H_2O (1/2, v/v)	100 °C; 1500 psi	3 x 5 min	100 %; 60 sec	Freeze dried sludge samples spiked and then stirred intensively for 24 hr	Absolute recovery[j]: 70 – 124% (12/31) 44 – 69% (13/31) <30% (6/31)	PLE extract (approx. 22 mL) made up to 500 mL; SPE - Oasis HLB (200 mg)	Radjenović et al. [5]

17[k]	Sediment (3 g)	Sediment samples were lyophilised, sieved (< 2 mm) and homogenised Samples stored at -20 °C	Sea sand; 22 mL extraction cell	H_2O	90 °C; 500 psi	3 x 7 min	100 %; 60 sec	Soil/sediment spiked and stirred for 30 min. Samples then equilibrated for 24 h	Absolute recovery[l]: 70 – 95% (12/17) 48 – 64% (4/17) 37% (1/17) Relative recovery 65 – 104% (17/17)	PLE extract (approx. 30 mL); SPE - Isolute SAX (500 mg) placed on top of an Oasis HLB (60 mg)	Vasquez-Roig et al. [6]
	Soil (3 g)	Soil samples were air dried in the dark at 20 °C, sieved (< 2 mm) and homogenised Samples stored at 4 °C							Absolute recovery[l]: 74 – 104% (10/17) 41 –64% (6/17) 37% (1/17) Relative recovery 67 – 108% (17/17)		
6[m]	SPM[n]	Waste water filtered through glass-fibre filters and filters lyophilised Frozen until analysis Filters sonicated in 100 mL 50 mM formic acid/MeOH (80/20, v/v) (repeated twice)	N/A	N/A	N/A	N/A	N/A	Analytes spiked on to 70 glass fibre filters	Absolute recovery[o]: 70 – 87% (6/6)	Sonicated sample made up to 2100 mL; SPE - Oasis MCX (pH 3)	Metcalfe et al. [7]

N.R. = not reported in manuscript; N/A = not applicable as PLE was not used in the analytical method; [a] Amphetamine; [b] 10 pharmaceuticals; [c] It is not clear in the manuscript how recoveries were calculated, although it would seem that spiked sludge was compared to spiked diluent; [d] 27 pharmaceuticals; [e] Recoveries calculated by spiking soil/sludge before PLE and comparing against blank soil/sludge spiked during reconstitution; [f] Recoveries for three compounds could not be calculated in sewage sludge; [g] 12 pharmaceuticals; [h] Absolute recoveries calculated by spiking sediment prior to PLE and comparing the peak area to a standard solution without matrix; [I] 31 pharmaceuticals; [j] Recoveries were determined through pre- and post spiking of matrix. However, it is not clear in this manuscript if recoveries were absolute or relative, and if they refer to only the PLE step or both PLE and SPE; [k] 17 pharmaceuticals; [l] Absolute recovery calculated by spiking soil and sediment before PLE and relates to the entire extraction process, although it is unclear if this value includes matrix effects; [m] 6 drugs of abuse; [n] Particulate matter on filters used for testing, but dry weight of SPM not reported; [o] Recovery calculated by spiking filters without matrix before USE, and comparing to standard solution

Table S2 - Selected analytes and their properties

Compound	CAS	Formula	MW	Pka		LogP		LogD[b]		Supplier
				Exp.[a]	Calc.[b]	Exp.[a]	Calc.[b]	pH 7	pH 8	
Stimulants and their metabolites										
Cocaine	50-36-2	$C_{17}H_{21}NO_4$	303.4	8.6 (20°)	8.9	2.3	2.3	0.3	1.3	LGC
Benzoylecgonine	519-09-5	$C_{16}H_{19}NO_4$	289.3	-	10.8, 3.3	-1.3	2.3	-0.2	-0.2	LGC
Norbenzoylecgonine	60426-41-7	$C_{15}H_{17}NO_4$	275.3	-	10.4, 3.4	-	2.6	0.1	0.1	LGC
Norcocaine	-	$C_{16}H_{19}NO_4$	289.3	-	9.0	-	3.1	1.1	2.1	LGC
Cocaethylene	529-38-4	$C_{18}H_{23}NO_4$	317.4	-	9.0	-	2.8	0.8	1.7	LGC
Anhydroecgonine methyl ester	43021-26-7	$C_{10}H_{15}NO_2$	181.2	-	8.0	-	0.4	0.1	-0.6	LGC
Ecgonidine	74242-55-0	$C_8H_{11}NO_2$	153.2	-	9.6, 3.8	-	1.5	-1	-1	LGC
Amphetamine	300-62-9	$C_9H_{13}N$	135.2	10.1	9.9	1.8	1.8	-0.9	-0.1	LGC
Methamphetamine	R-(-):33817-09-3, S-(+):537-46-2	$C_{10}H_{15}N$	149.2	10.1	10.4	2.1	2.2	-0.7	-0.1	LGC
Methcathinone	49656-78-2	$C_{10}H_{13}NO$	163.2	-	7.1	-	0.4	0	0.3	Sigma-Aldrich
BZP	-	$C_{11}H_{16}N_2$	176.3	-	9.3, 3.4	-	1.1	-1	-0.1	LGC
TFMPP	-	$C_{11}H_{13}F_3N_2$	230.2	-	8.8, 2.1	-	1.3	0.7	-0.2	LGC
Hallucinogens and their metabolites										
MDA	4764-17-4	$C_{10}H_{13}NO_2$	179.2	-	9.9	1.64	1.6	-1.1	-0.3	LGC
MDMA	4254210-9	$C_{11}H_{15}NO_2$	193.2	(benzene, pH 9.0) 9.4	10.3	-	2.1	-0.8	-0.2	LGC
MDEA	82801-81-8	$C_{12}H_{17}NO_2$	207.3	-	10.3	-	2.6	-0.3	0.3	LGC
MBDB	145225-00-9	$C_{12}H_{17}NO_2$	207.3	-	10.5	-	2.6	-0.4	0.2	LGC
BDB	-	$C_{11}H_{15}NO_2$	193.2	-	10	-	2.2	-0.6	0.2	LGC
Mescaline	832-92-8	$C_{11}H_{17}NO_3$	211.3	9.6	9.6	0.8	0.5	-1.9	-1	LGC
LSD	50-37-3	$C_{20}H_{25}N_3O$	323.4	7.5	7.4	2.9	2.7	2.1	2.6	LGC
O-H-LSD	-	$C_{20}H_{25}N_3O_3$	355.4	-	11.7, 6.8	-	-1.9	-2.1	-1.9	LGC
Human indicators										
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	14.0 (25°), 10.4 (40°)	0.5	-0.07	-0.6	-0.6	-0.6	Sigma-Aldrich
1,7-dimethylxanthine	611-59-6	$C_7H_8N_4O_2$	180.2	-	8.5, 0.2	-	-0.9	-1	-1.1	Sigma-Aldrich
Nicotine	54-11-5	$C_{10}H_{14}N_2$	162.2	7.9, 3.2, (25°)	8.0, 3.2	1.2	0.6	-1	-0.1	Sigma-Aldrich
Continine	486-56-6	$C_{10}H_{12}N_2O$	176.2	-	4.7	-	0.07	0.1	0.1	Sigma-Aldrich
Opioids, morphine derivatives and their metabolites										
Heroin	561-27-3	$C_{21}H_{23}NO_5$	369.4	7.6 (23°)	7.9	1.58	1.6	0.6	1.3	LGC
6-acetylmorphine	2784-73-8	$C_{19}H_{21}NO_4$	327.4	-	9.4, 8.0	-	1.6	0.6	1.3	LGC
Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.4	8.2 (20°)	13.4, 8.2	0.6	1.4	0.1	0.9	Sigma-Aldrich
Norcodeine	467-15-2	$C_{17}H_{19}NO_3$	285.3	9.2 (25°)	13.3, 9.3	0.7	0.5	-1.8	-0.9	LGC
Oxycodone	76-42-6	$C_{18}H_{21}NO_4$	315.4	8.9 (20°)	13.1, 7.6	0.7	1.6	0.8	1.4	LGC
Oxymorphone	76-41-5	$C_{17}H_{19}NO_4$	301.3	9.3, 8.5	13.5, 9.2, 7.6	0	1.2	0.5	1	LGC
Morphine	57-27-2	$C_{17}H_{19}NO_3$	285.3	9.9, 8.0 (20°)	13.5, 9.5, 8.3	-0.1	0.9	-0.3	0.5	LGC
Normorphine	466-97-7	$C_{16}H_{17}NO_3$	271.3	9.8 (25°)	13.4, 9.5, 9.2	-2.8	0	-2.3	-1.4	LGC
Dihydrocodeine	125-28-0	$C_{18}H_{23}NO_3$	301.4	8.8 (25°)	8.4	-	0.6	-0.9	0	LGC
Buprenorphine	52485-79-7	$C_{29}H_{41}NO_4$	467.6	8.5, 10.0	9.5, 8.3	5	2.8	1.5	2.3	LGC
Norbuprenorphine	78715-23-8	$C_{25}H_{35}NO_4$	413.6	-	9.8, 9.1	-	1.2	-1.2	-0.4	LGC
Methadone	76-99-3	$C_{21}H_{27}NO$	309.4	8.94 (25°), 8.3 (20°)	9.1	3.9	3.9	1.9	2.8	Sigma-Aldrich
EDDP	66729-78-0	$C_{21}H_{25}N$	291.4	-	8.4	-	5	3.7	4.5	LGC
EMDP	-	$C_{20}H_{23}N$	277.4	-	8.1	-	5.8	4.7	5.4	LGC
Fentanyl	437-38-7	$C_{22}H_{28}N_2O$	336.5	-	8.9, 0.3	2.3	3.7	2.2	3.1	LGC
Norfentanyl	-	$C_{14}H_{20}N_2O$	232.3	-	9.8, 0.3	-	1.7	-0.8	0.1	LGC
Propoxyphene	469-62-5	$C_{22}H_{29}NO_2$	339.5	6.3	9.2	4.2	4.1	1.9	2.9	LGC
Norpropoxyphene	159208-83-0	$C_{21}H_{27}NO_2$	325.4	-	10.1	-	3.7	0.9	1.7	LGC
Tramadol	36282-47-0	$C_{16}H_{25}NO_2$	263.4	9.4, 8.3	9.6	3	2.3	-0.1	0.9	Sigma-Aldrich
Nortramadol	-	$C_{15}H_{23}NO_2$	249.4	-	10.6	-	1.7	-1.3	-0.7	LGC
Benzodiazepines and their metabolites										
Temazepam	846-50-4	$C_{16}H_{13}ClN_2O_2$	300.7	1.6	11.7, 1.6	2.2	2.2	2.2	2.2	LGC
Diazepam	439-15-5	$C_{16}H_{13}ClN_2O$	284.7	3.3 (20°)	3.4	2.7	2.8	2.8	2.8	LGC
Nordiazepam	1088-11-5	$C_{15}H_{11}ClN_2O$	270.7	12.0, 3.5	11.7, 3.2	2.9	2.8	2.8	2.8	LGC
Nitrazepam	146-22-5	$C_{15}H_{11}N_3O_3$	281.3	10.8, 3.2 (20°)	11.4, 2.6	2.1	2.4	2.4	2.4	Sigma-Aldrich
7-aminonitrazepam	4928-02-3	$C_{15}H_{13}N_3O$	251.3		12.3, 4.3, 2.3	-	1.1	1.1	1.1	LGC
Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.7	11.6, 1.7 (20°)	12.8, 10.9, 1.2	2.2	2.2	2.2	2.2	LGC
Chlordiazepoxide	58-25-3	$C_{16}H_{14}ClN_3O$	299.8	4.8	8.6, 6.5	2.4	2.8	2.2	2.1	LGC
Antidepressants and their metabolites										
Dosulepin	113-53-1	$C_{19}H_{21}NS$	295.4	-	9.1	2.8	4.3	2.2	3.1	LGC
Amitriptyline	549-18-8	$C_{20}H_{23}N$	277.4	9.4 (25°)	9.2	5	4.4	2.3	3.2	Sigma-Aldrich
Nortriptyline	894-71-3	$C_{19}H_{21}N$	263.4	9.7	10	1.7	4	1.2	2	Sigma-Aldrich
Fluoxetine	59333-67-4	$C_{17}H_{18}F_3NO$	309.3	-	10.1	1.8	3.9	1.2	1.9	LGC

Norfluoxetine	-	$C_{16}H_{16}F_3NO$	295.3	-	9.1	-	3.8	1.7	2.7	LGC
Venlafaxine	99300-78-4	$C_{17}H_{27}NO_2$	277.4	-	9.3	0.4	2.5	0.4	1.3	Sigma-Aldrich
Dissociative anesthetics and their metabolites										
Phencyclidine	77-10-1	$C_{17}H_{25}N$	243.4	8.5	8.2	4.7	4.3	3	3.8	LGC
Ketamine	1867-66-9	$C_{13}H_{16}ClNO$	237.7	7.5	6.5	3.1	3	2.9	3	Sigma-Aldrich
Norketamine	-	$C_{12}H_{14}ClNO$	223.7	6.7	6.3		2.4	2.3	2.3	LGC
Other										
Methaqualone	72-44-6	$C_{16}H_{14}N_2O$	250.3	2.5	3	4.3	2.5	2.5	2.5	LGC
Sildenafil	139755-83-23	$C_{22}H_{30}N_6O_4S$	474.6	8.7	6.0, 0.6		1.6	1.6	1.6	LGC
Drug precursors										
Ephedrine	50-98-6	$C_{10}H_{15}NO$	165.2	9.6 (25°)	9.5	1.1	1	-1.3	-0.4	LGC
Norephedrine	154-41-6	$C_9H_{13}NO$	151.2	-	12.1, 8.5		0.4	-1.9	-1	Sigma-Aldrich

[a] extracted from [8]

[b] predicated using ACD labs software [9]

Table S3 - Optimized MRM conditions and ion ratios

Compound	CV/CE[a]	MRM1 (quantification)	CV/CE[a]	MRM2 (confirmation)	MRM ratio[b] ± % RSD
Stimulants and their metabolites					
Cocaine	40/20	304.2 > 182.1	40/31	304.2 > 82.1	2.56 ± 0.04
Benzoylecgonine	38/19	290.2 > 168.1	38/30	290.2 > 105.1	2.67 ± 0.04
Norbenzoylecgonine	32/16	276.1 > 154.0	32/21	276.1 > 136.1	1.53 ± 0.03
Norcocaine	40/15	290.2 > 168.1	40/24	290.1 > 136.1	1.76 ± 0.10
Cocaethylene	38/20	318.2 > 196.2	38/30	318.2 > 82.1	1.68 ± 0.03
Anhydroecgonine methyl ester	39/23	182.1 > 118.0	39/21	182.1 > 122.1	0.83 ± 0.09
Ecgonidine	37/23	168.1 > 91.1	37/20	168.1 > 122.1	2.68 ± 0.22
Amphetamine	18/8	136.2 > 119.1	18/16	136.2 > 91.1	4.55 ± 0.16
Methamphetamine	24/19	150.2 > 91.1	24/10	150.2 > 119.1	2.82 ± 0.11
Methcathinone	28/19	164.1 > 131.0	28/12	164.1 > 146.1	0.55 ± 0.03
BZP	35/20	177.1 > 91.1	35/15	177.1 > 85.1	6.18 ± 0.11
TFMPP	46/23	231.0 > 188.0	46/35	231.0 > 118.3	2.42 ± 0.06
Hallucinogens and their metabolites					
MDA	21/11	180.0 > 163.1	21/22	180.0 > 105.1	3.05 ± 0.07
MDMA	24/13	194.1 > 163.1	24/24	194.1 > 105.1	2.51 ± 0.08
MDEA	28/13	208.1 > 163.1	28/27	208.1 > 105.1	3.18 ± 0.05
MBDB	26/20	208.1 > 135.1	26/11	208.1 > 177.1	2.52 ± 0.14
BDB	20/9	194.1 > 177.1	20/16	194.1 > 135.1	4.03 ± 0.51
Mescaline	46/12	212.3 > 195.1	46/18	212.3 > 180.1	1.83 ± 0.15
LSD	41/24	324.2 > 223.2	41/28	324.2 > 208.1	1.62 ± 0.04
O-H-LSD	41/24	356.2 > 237.1	41/24	356.2 > 74.1	1.46 ± 0.04
Human indicators					
Caffeine	38/15	195.1 > 138.0	38/23	195.1 > 110.0	2.93 ± 0.06
1,7-dimethylxanthine	54/21	181.0 > 124.1	none	none	- -
Nicotine	37/20	163.1 > 130.0	37/24	163.1 > 117.0	0.95 ± 0.04
Continine	34/21	177.1 > 80.0	34/22	177.1 > 98.1	3.15 ± 0.08
Creatinine	31/11	114.0 > 86.1	31/16	114.0 > 72.1	2.54 ± 0.08
Opioids, morphine derivatives and their metabolites					
Heroin	51/50	370.2 > 165.1	51/29	370.2 > 268.1	1.39 ± 0.04
6-acetylmorphine	52/39	328.1 > 165.1	52/26	328.1 > 211.1	1.77 ± 0.04
Codeine	49/57	300.2 > 152.1	49/25	300.2 > 215.1	1.22 ± 0.03
Norcodeine	46/40	286.1 > 165.1	46/20	286.1 > 268.2	0.87 ± 0.03
Oxycodone	36/29	316.2 > 241.1	36/26	316.2 > 256.1	1.46 ± 0.04
Oxymorphone	40/19	302.1 > 284.1	40/28	302.1 > 227.1	3.59 ± 0.09
Morphine	53/56	286.1 > 152.1	53/38	286.1 > 165.1	1.33 ± 0.05
Normorphine	45/49	272.1 > 152.1	45/43	272.1 > 165.0	1.34 ± 0.03
Dihydrocodeine	53/33	302.1 > 199.1	53/60	302.1 > 128.1	1.48 ± 0.04
Buprenorphine	69/45	468.3 > 84.1	69/43	468.3 > 101.0	1.69 ± 0.04
Norbuprenorphine	60/47	414.3 > 83.0	60/39	414.3 > 101.1	1.26 ± 0.03
Methadone	31/15	310.2 > 265.1	31/28	310.2 > 105.1	2.26 ± 0.08
EDDP	50/29	278.2 > 234.1	50/24	278.2 > 249.1	1.15 ± 0.11
EMDP	47/21	264.2 > 235.1	47/31	264.2 > 220.2	1.04 ± 0.02

Fentanyl	44/38	337.2 > 105.1	44/23	337.2 > 188.2	1.01	±	0.03
Norfentanyl	27/20	233.2 > 84.0	27/15	233.2 > 177.1	17.68	±	0.44
Propoxyphene	19/8	340.2 > 266.2	19/22	340.2 > 143.0	24.40	±	1.28
Norpropoxyphene	15/7	326.2 > 252.1	none	none	-		-
Tramadol	24/17	264.2 > 58.1	24/11	264.2 > 246.3	92.34	±	3.07
Nortramadol	21/8	250.2 > 232.1	none	none	-	-	-
Benzodiazepines and their metabolites							
Temazepam	37/21	301.1 > 255.1	37/14	301.1 > 283.1	1.97	±	0.07
Diazepam	54/27	285.0 > 154.1	54/31	285.0 > 193.1	1.32	±	0.02
Nordiazepam	51/29	271.1 > 140.1	51/29	271.1 > 165.0	1.26	±	0.06
Nitrazepam	44/24	282.1 > 236.1	44/37	282.1 > 180.1	6.12	±	0.29
7-aminonitrazepam	48/25	252.1 > 121.1	48/40	252.1 > 94.1	3.05	±	0.09
Oxazepam	38/21	287.1 > 241.1	38/15	287.1 > 269.0	1.15	±	0.04
Chlordiazepoxide	32/15	300.1 > 283.1	32/25	300.1 > 227.1	2.04	±	0.08
Antidepressants and their metabolites							
Dosulepin	34/24	296.1 > 218.1	34/24	296.1 > 223.1	0.81	±	0.02
Amitriptyline	37/26	278.2 > 91.1	37/18	278.2 > 233.2	1.10	±	0.02
Nortriptyline	33/16	264.2 > 233.1	33/23	264.2 > 91.0	1.30	±	0.03
Fluoxetine	25/8	310.3 > 148.1	none	none	-		-
Norfluoxetine	17/7	296.2 > 134.1	none	none	-		-
Venlafaxine	27/12	278.2 > 260.1	27/32	278.2 > 121.0	3.93	±	0.11
Dissociative anaesthetics and their metabolites							
Phencyclidine	18/14	244.2 > 159.1	18/34	244.2 > 91.1	1.24	±	0.03
Ketamine	31/27	238.1 > 125.0	31/15	238.1 > 220.1	2.71	±	0.04
Norketamine	23/27	224.0 > 125.0	23/12	224.0 > 207.1	1.02	±	0.03
Other							
Methaqualone	58/27	251.1 > 132.1	58/42	251.1 > 91.1	5.53	±	0.59
Sildenafil	60/28	475.3 > 100.2	60/40	475.3 > 283.2	4.46	±	0.07
Drug precursors							
Ephedrine/Pseudoephedrine	23/12	166.1 > 148.1	23/21	166.1 > 133.0	43.37	±	5.41
Norephedrine	21/11	152.1 > 134.1	21/19	152.1 > 117.1	64.26	±	7.56
Internal standards							
Cocaine-D3	40/20	307.2 > 185.1					
Benzoylecgonine-D8	38/19	298.2 > 171.1					
Cocaethylene-D8	38/20	326.2 > 204.2					
Amphetamine-D11	18/8	147.2 > 130.1					
Methamphetamine-D14	24/19	164.2 > 98.1					
MDA-D5	21/11	185.1 > 168.1					
MDMA-D5	26/13	199.1 > 165.1					
MBDB-D5	26/20	213.1 > 136.1					
MDEA-D5	28/13	213.1 > 163.0					
Caffeine-D9	38/15	204.2 > 144.1					

Nicotine-D4	37/20	167.1 > 134.1
LSD-D3	41/24	327.2 > 226.1
Heroin-D9	51/50	379.2 > 165.8
Oxycodone-D6	36/29	322.2 > 247.1
Morphine-D6	53/38	292.2 > 171.1
Methadone-D9	31/15	319.3 > 268.2
EDDP-D3	50/29	281.2 > 234.1
Fentanyl-D5	44/38	342.2 > 105.1
Codeine-D6	52/28	306.2 > 218.1
Dihydrocodeine-D6	53/33	308.2 > 202.1
Buprenorphine-D4	69/45	472.4 > 88.1
Propoxyphene-D11	19/8	351.3 > 277.2
Norpropoxyphene-D5	15/7	331.2 > 257.2
Temazepam-D5	37/21	306.7 > 260.1
Diazepam-D5	54/27	290.1 > 154.1
Oxazepam-D5	38/21	292.0 > 246.0
Fluoxetine-D6	25/8	316.2 > 154.1
PCP-D5	18/14	249.2 > 164.1
Ketamine-D4	31/27	242.1 > 129.1
Mescaline-D9	46/12	221.2 > 204.2
Methaqualone-D7	58/27	258.2 > 139.1

[a]CV, cone voltage (V); CE, collision energy (eV)
[b] MRM ratio : MRM1/MRM2 ratio calculated in surface water

Effect of sample methanol content on SPE recovery

Initially, before optimising the PLE process, an investigation into the percentage of organic solvent that can be used before effecting SPE recoveries was undertaken. This parameter is of importance due to the need to dilute PLE extracts to reduce organic composition [3,5,10,11]. Dilution is generally performed so that organic content is around 5 % or less. However, doing so then results in increased times for SPE extraction.

In this study the percentage of methanol in water was investigated at 25, 50 and 75 %. All analytes were spiked into 100 mL of methanol: water at 10 ng per analyte and extracted in duplicate according to the SPE procedure described in section 2.4. The recoveries for each analyte in the different solvents were normalised against the recovery generated from a completely aqueous sample. Results are shown in Table S4.

The results show that a sample composition of 25 % methanol in relation to 0 % methanol leads to little effect on the vast majority of analytes, with a change of less than 10 % for nearly all compounds. However there were significant exceptions. Anhydroecgonine methyl ester and ecgonidine reported a decrease in recovery of 63 and 77 %, respectively. Similarly, a high decrease in recovery was observed for caffeine (94 %), 1,7-dimethylxanthine (94 %), nicotine (93 %) and continine (86 %). A percentage of methanol content of 50 and 75 % resulted in unacceptably decreased recoveries for nearly all compounds.

Although a methanol composition of 25 % led to the significant decrease in the recovery of a small number of compounds, it was decided that the ability to use a much smaller dilution factor was more beneficial. In particular this was because the analytes in question, anhydroecgonine methyl ester and ecgonidine, had not been measured at > MDL in wastewater between the sampling months of December 2009 to November 2010. Therefore it was unlikely that either compound would be detected in SPM. Caffeine, 1,7-dimethylxanthine, nicotine and continine were not further analysed due to low SPE recovery.

The ability to use up to 25 % methanol in water when performing SPE with an Oasis MCX may provide an important advantage over methods which employ the Oasis HLB and need to dilute methanol content to < 5 %. For instance Jelic et al. [11] used a solvent of methanol/ water, 1/2 (v/v) and obtained a PLE extract of around 22 mL which was diluted to 500 mL and extracted using an Oasis HLB sorbent. With the SPE method in this study it may have been possible to dilute to a much smaller volume of around 60 mL. Similarly, Barron et al. [3] used a PLE solvent of methanol water, 1/1 (v/v) and obtained a PLE extract of around 53 mL which was then diluted to 1000 mL and extracted with Oasis HLB sorbent. Using the SPE method in this study it may have been possible to dilute to just over 100 mL. These conclusions are of course speculative, and with a different set of compounds the recoveries obtained may have changed dramatically. Nevertheless, this shows that a mixed mode sorbent may provide significant improvements in reducing the dilution factor and in turn the time required for extraction. When selecting SPE sorbent, an evaluation of the effect of methanol content should be considered rather than simply the recovery of compounds in isolation.

Table S4 – SPE recovery with an increasing methanol to water sample composition

Compound	SPE recovery (n=2, 100ng L^{-1})		
	SPE recovery change (%) in relation to 0:100 methanol:water		
	25:75	50:50	75:25
Stimulants			
Cocaine	-1 ± 1	-18 ± 1	-76 ± 1
Benzoylecgonine	2 ± 1	-56 ± 0	-89 ± 0
Norbenzoylecgonine	4 ± 3	-27 ± 1	-83 ± 0
Norcocaine	-3 ± 0	-10 ± 1	-65 ± 2
Cocaethylene	-1 ± 0	-12 ± 3	-76 ± 1
Anhydroecgonine methyl ester	-63 ± 9	-79 ± 2	-88 ± 3
Ecgonidine	-77 ± 2	-89 ± 1	-90 ± 2
Amphetamine	-3 ± 2	-9 ± 0	-63 ± 0
Methamphetamine	-5 ± 3	-4 ± 7	-36 ± 5
Methcathinone	-17 ± 7	-37 ± 5	-77 ± 3
BZP	-3 ± 5	-15 ± 4	-14 ± 7
TFMPP	-2 ± 0	-9 ± 2	-12 ± 1
Hallucinogens			
MDA	-1 ± 4	4 ± 5	2 ± 6
MDMA	-1 ± 3	18 ± 17	26 ± 16
MDEA	1 ± 4	10 ± 7	12 ± 1
MBDB	0 ± 6	-1 ± 9	-26 ± 4
BDB	0 ± 5	-3 ± 3	-41 ± 1
Mescaline	4 ± 5	-43 ± 3	-76 ± 0
LSD	-3 ± 1	-11 ± 6	-36 ± 2
O-H-LSD	-15 ± 4	-75 ± 1	-91 ± 0
Human indicators			
Caffeine	-94 ± 0	-97 ± 0	-98 ± 0
1,7-dimethylxanthine	-94 ± 1	-98 ± 0	-99 ± 0
Nicotine	-93 ± 1	-86 ± 7	-92 ± 0
Continine	-86 ± 4	-90 ± 2	-87 ± 5
Opioids and morphine derivatives			
Heroin	-3 ± 2	-37 ± 1	-80 ± 0
6-acetylmorphine	-3 ± 1	-54 ± 1	-80 ± 1
Codeine	3 ± 6	-51 ± 1	-74 ± 0

Norcodeine	5 ± 2	-36 ± 4	-64 ± 0
Oxycodone	-5 ± 5	-59 ± 1	-83 ± 2
Oxymorphone	2 ± 3	-74 ± 1	-82 ± 2
Morphine	-5 ± 6	-74 ± 0	-81 ± 3
Normorphine	-4 ± 4	-69 ± 3	-77 ± 2
Dihydrocodeine	0 ± 5	-60 ± 0	-80 ± 0
Buprenorphine	0 ± 1	-14 ± 13	-66 ± 3
Norbuprenorphine	-2 ± 0	-15 ± 4	-61 ± 3
Methadone	-2 ± 2	-14 ± 5	-60 ± 4
EDDP	3 ± 5	6 ± 13	-17 ± 5
EMDP	-11 ± 7	15 ± 10	-44 ± 5
Fentanyl	-1 ± 0	-38 ± 2	-77 ± 1
Norfentanyl	0 ± 1	-5 ± 2	-33 ± 2
Propoxyphene	-2 ± 2	-20 ± 6	-66 ± 4
Norpropoxyphene	-6 ± 3	-28 ± 5	-51 ± 7
Tramadol	-2 ± 0	-32 ± 0	-80 ± 1
Nortramadol	-1 ± 8	-11 ± 1	-72 ± 1
Benzodiazepines			
Temazepam	3 ± 6	-89 ± 0	-99 ± 0
Diazepam	4 ± 4	-10 ± 1	-77 ± 2
Nordiazepam	7 ± 5	-16 ± 1	-76 ± 1
Nitrazepam	-6 ± 1	-41 ± 4	-84 ± 2
7-aminonitrazepam	0 ± 1	-18 ± 12	-18 ± 1
Oxazepam	-4 ± 0	-89 ± 1	-99 ± 0
Chlordiazepoxide	-4 ± 3	-26 ± 4	-54 ± 3
Antidepressants			
Dosulepin	4 ± 4	-10 ± 13	-12 ± 1
Amitriptyline	-1 ± 0	-10 ± 6	-15 ± 2
Nortriptyline	5 ± 8	4 ± 10	12 ± 19
Fluoxetine	9 ± 1	1 ± 3	6 ± 8
Norfluoxetine	7 ± 3	-1 ± 7	5 ± 5
Venlafaxine	3 ± 1	-13 ± 2	-68 ± 1
Dissociative anaesthetics			
Phencyclidine	-7 ± 1	-9 ± 4	-55 ± 1
Ketamine	-6 ± 11	-38 ± 6	-83 ± 0

Norketamine	-8 ± 0	-61 ± 1	-90 ± 1
Other			
Methaqualone	-7 ± 2	-7 ± 2	-83 ± 2
Sildenafil	5 ± 3	-10 ± 17	-14 ± 7
Drug precursors			
Ephedrine	-2 ± 3	-57 ± 1	-81 ± 0
Norephedrine	2 ± 3	-60 ± 1	-84 ± 0

Table S5 – Absolute PLE recoveries with different solvents (uppermost recovery highlighted)

Compound	PLE recovery (%) (n = 2, spiked amount 150 ng g^{-1})				
	Methanol / water (pH 2)				
	1 / 3	1 / 2	1 / 1	2 / 1	3 / 1
Stimulants					
Cocaine	82 ± 0	85 ± 2	88 ± 2	82 ± 4	79 ± 3
Benzoylecgonine	100 ± 1	92 ± 1	95 ± 4	91 ± 9	88 ± 4
Norbenzoylecgonine	100 ± 2	91 ± 1	97 ± 4	84 ± 9	82 ± 2
Norcocaine	95 ± 4	95 ± 2	91 ± 0	82 ± 4	81 ± 1
Cocaethylene	86 ± 1	90 ± 2	92 ± 2	88 ± 4	87 ± 2
Amphetamine	92 ± 11	86 ± 4	78 ± 2	72 ± 1	76 ± 3
Methamphetamine	107 ± 3	95 ± 0	93 ± 1	82 ± 1	90 ± 3
Methcathinone	124 ± 13	122 ± 2	113 ± 8	81 ± 1	85 ± 8
BZP	0 ± 0	1 ± 1	0 ± 0	0 ± 0	ND ± 0
TFMPP	66 ± 3	75 ± 3	81 ± 2	73 ± 4	77 ± 0
Hallucinogens					
MDA	97 ± 5	90 ± 2	93 ± 3	87 ± 3	91 ± 5
MDMA	104 ± 4	94 ± 1	94 ± 3	86 ± 8	92 ± 4
MDEA	107 ± 5	99 ± 1	97 ± 1	84 ± 4	88 ± 6
MBDB	96 ± 2	94 ± 1	94 ± 0	89 ± 5	87 ± 3
BDB	93 ± 5	89 ± 2	89 ± 2	82 ± 3	85 ± 1
Mescaline	93 ± 2	94 ± 0	92 ± 1	85 ± 4	96 ± 2
LSD	67 ± 2	73 ± 1	87 ± 3	77 ± 6	66 ± 2
O-H-LSD	110 ± 2	107 ± 1	93 ± 1	70 ± 5	63 ± 3
Opiods and morphine derivatives					
Heroin	109 ± 3	107 ± 2	109 ± 3	92 ± 8	85 ± 1
6-acetylmorphine	79 ± 4	78 ± 2	80 ± 4	72 ± 3	76 ± 1
Codeine	94 ± 4	94 ± 0	93 ± 3	81 ± 3	75 ± 0
Norcodeine	95 ± 4	95 ± 2	95 ± 1	84 ± 6	78 ± 1
Oxycodone	91 ± 4	94 ± 1	90 ± 1	75 ± 3	74 ± 1
Oxymorphone	77 ± 4	86 ± 2	87 ± 1	69 ± 1	63 ± 0
Morphine	69 ± 2	71 ± 0	76 ± 4	76 ± 1	67 ± 0
Normorphine	70 ± 2	73 ± 4	77 ± 4	86 ± 0	79 ± 1
Dihydrocodeine	90 ± 1	89 ± 0	88 ± 3	75 ± 2	69 ± 1

Buprenorphine	2 ± 0	7 ± 2	40 ± 3	58 ± 4	60 ± 2
Norbuprenorphine	49 ± 0	58 ± 2	78 ± 5	67 ± 4	62 ± 2
Methadone	78 ± 2	86 ± 0	93 ± 1	83 ± 7	79 ± 0
EDDP	51 ± 3	58 ± 3	82 ± 4	69 ± 8	65 ± 2
EMDP	92 ± 4	80 ± 4	89 ± 4	92 ± 2	91 ± 0
Fentanyl	0 ± 0	0 ± 0	1 ± 0	1 ± 0	1 ± 0
Norfentanyl	73 ± 0	74 ± 3	81 ± 0	70 ± 3	71 ± 0
Propoxyphene	69 ± 11	78 ± 3	84 ± 3	71 ± 7	73 ± 1
Norpropoxyphene	62 ± 12	76 ± 1	87 ± 5	69 ± 8	69 ± 1
Tramadol	101 ± 4	96 ± 1	95 ± 1	87 ± 4	87 ± 5
Nortramadol	88 ± 9	76 ± 3	73 ± 5	61 ± 5	65 ± 2
Benzodiazepines					
Temazepam	31 ± 2	39 ± 3	84 ± 15	101 ± 5	68 ± 3
Diazepam	80 ± 5	101 ± 0	99 ± 1	93 ± 5	85 ± 1
Nordiazepam	56 ± 13	75 ± 0	84 ± 5	82 ± 6	28 ± 2
Nitrazepam	64 ± 18	77 ± 1	81 ± 4	66 ± 5	22 ± 0
7-aminonitrazepam	3 ± 1	5 ± 3	16 ± 3	6 ± 2	1 ± 0
Oxazepam	67 ± 2	67 ± 1	104 ± 3	142 ± 15	139 ± 16
Chlordiazepoxide	101 ± 1	101 ± 0	105 ± 2	93 ± 9	81 ± 3
Antidepressants					
Dosulepin	65 ± 2	77 ± 0	87 ± 3	73 ± 10	68 ± 1
Amitriptyline	65 ± 4	76 ± 2	88 ± 6	74 ± 10	67 ± 1
Nortriptyline	82 ± 1	89 ± 3	100 ± 4	80 ± 10	69 ± 1
Fluoxetine	23 ± 1	49 ± 2	84 ± 5	69 ± 10	67 ± 4
Norfluoxetine	42 ± 5	65 ± 1	88 ± 5	65 ± 10	57 ± 0
Venlafaxine	36 ± 1	47 ± 4	68 ± 3	61 ± 7	61 ± 3
Dissociative anaesthetics					
Phencyclidine	4 ± 0	8 ± 2	29 ± 2	27 ± 3	29 ± 0
Ketamine	109 ± 1	103 ± 0	98 ± 2	94 ± 0	99 ± 1
Norketamine	105 ± 5	102 ± 0	102 ± 2	91 ± 2	97 ± 3
Other					
Methaqualone	88 ± 2	87 ± 1	91 ± 3	88 ± 4	77 ± 2
Sildenafil	57 ± 4	74 ± 5	94 ± 9	62 ± 7	46 ± 2

Drug precursors					
Ephedrine	91 ± 1	88 ± 1	83 ± 1	68 ± 3	69 ± 0
Norephedrine	95 ± 9	89 ± 0	77 ± 1	65 ± 4	56 ± 3

Table S6 – Absolute PLE recoveries at different temperatures

Compound	PLE recovery (%) (n = 2, spiked amount 150 ng g^{-1})				
	Extraction temperature				
	40°C	60°C	80°C	100°C	120°C
Stimulants					
Cocaine	57 ± 1	81 ± 2	86 ± 1	91 ± 1	95 ± 0
Benzoylecgonine	90 ± 1	96 ± 2	94 ± 3	96 ± 3	101 ± 2
Norbenzoylecgonine	92 ± 0	94 ± 3	97 ± 3	93 ± 4	87 ± 2
Norcocaine	85 ± 0	95 ± 1	89 ± 0	94 ± 3	100 ± 0
Cocaethylene	75 ± 0	91 ± 0	88 ± 2	93 ± 1	96 ± 3
Amphetamine	98 ± 3	87 ± 3	82 ± 3	88 ± 1	89 ± 3
Methamphetamine	90 ± 5	85 ± 4	84 ± 5	93 ± 2	95 ± 1
Methcathinone	120 ± 1	119 ± 0	97 ± 5	110 ± 1	79 ± 9
BZP	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
TFMPP	71 ± 2	89 ± 0	89 ± 3	78 ± 5	45 ± 9
Hallucinogens					
MDA	85 ± 5	85 ± 6	93 ± 2	95 ± 2	92 ± 1
MDMA	73 ± 6	77 ± 8	88 ± 5	94 ± 2	93 ± 1
MDEA	77 ± 8	83 ± 3	93 ± 0	98 ± 1	102 ± 1
MBDB	88 ± 1	91 ± 2	90 ± 0	95 ± 2	97 ± 0
BDB	87 ± 3	87 ± 3	86 ± 1	88 ± 1	90 ± 2
Mescaline	90 ± 3	89 ± 1	89 ± 1	88 ± 2	80 ± 3
LSD	89 ± 2	93 ± 0	85 ± 4	71 ± 4	45 ± 1
O-H-LSD	94 ± 1	91 ± 2	85 ± 2	81 ± 3	79 ± 3
Opiods and morphine derivatives					
Heroin	54 ± 1	69 ± 1	74 ± 1	71 ± 3	67 ± 11
6-acetylmorphine	94 ± 2	92 ± 1	83 ± 0	79 ± 1	77 ± 2
Codeine	89 ± 1	94 ± 0	88 ± 1	87 ± 1	82 ± 0
Norcodeine	96 ± 0	97 ± 3	90 ± 2	89 ± 3	83 ± 1
Oxycodone	90 ± 2	93 ± 1	87 ± 0	84 ± 2	72 ± 1
Oxymorphone	91 ± 7	92 ± 9	79 ± 0	76 ± 9	57 ± 3
Morphine	82 ± 4	84 ± 6	78 ± 1	72 ± 4	57 ± 2
Normorphine	63 ± 4	74 ± 6	79 ± 2	68 ± 2	47 ± 3
Dihydrocodeine	83 ± 1	88 ± 1	85 ± 0	85 ± 1	83 ± 1
Buprenorphine	11 ± 0	21 ± 1	39 ± 3	42 ± 0	21 ± 7
Norbuprenorphine	63 ± 1	79 ± 2	82 ± 5	66 ± 7	28 ± 10
Methadone	86 ± 1	96 ± 2	94 ± 3	90 ± 4	86 ± 1
EDDP	24 ± 4	52 ± 2	77 ± 4	85 ± 1	89 ± 0
EMDP	71 ± 3	77 ± 3	87 ± 4	103 ± 4	96 ± 2
Fentanyl	0 ± 0	0 ± 0	1 ± 0	3 ± 1	30 ± 1
Norfentanyl	40 ± 1	62 ± 1	76 ± 0	83 ± 0	87 ± 0
Propoxyphene	72 ± 2	87 ± 3	85 ± 3	81 ± 4	87 ± 4
Norpropoxyphene	82 ± 2	91 ± 1	88 ± 4	89 ± 2	128 ± 7
Tramadol	89 ± 1	91 ± 1	87 ± 2	90 ± 2	93 ± 2
Nortramadol	93 ± 8	94 ± 2	87 ± 0	93 ± 2	102 ± 4
Benzodiazepines					
Temazepam	60 ± 1	76 ± 8	92 ± 3	99 ± 3	77 ± 7
Diazepam	104 ± 3	103 ± 1	97 ± 0	92 ± 4	81 ± 5
Nordiazepam	80 ± 1	84 ± 1	83 ± 1	74 ± 1	61 ± 4
Nitrazepam	74 ± 0	82 ± 2	64 ± 2	57 ± 1	50 ± 5

7-aminonitrazepam	0	±	0	1	±	0	9	±	2	19	±	0	27	±	4
Oxazepam	39	±	0	54	±	10	78	±	9	70	±	13	26	±	5
Chlordiazepoxide	83	±	0	92	±	5	84	±	2	66	±	3	31	±	6
Antidepressants															
Dosulepin	81	±	2	93	±	5	91	±	5	76	±	6	52	±	3
Amitriptyline	81	±	1	98	±	1	96	±	2	85	±	9	62	±	3
Nortriptyline	75	±	10	88	±	2	100	±	1	89	±	8	60	±	6
Fluoxetine	43	±	2	79	±	0	95	±	1	83	±	9	61	±	9
Norfluoxetine	63	±	1	89	±	1	97	±	4	79	±	11	57	±	8
Venlafaxine	20	±	0	42	±	0	64	±	6	74	±	2	95	±	3
Dissociative anaesthetics															
Phencyclidine	3	±	0	9	±	0	27	±	5	42	±	5	74	±	0
Ketamine	91	±	2	90	±	1	92	±	3	100	±	2	100	±	1
Norketamine	94	±	4	90	±	4	93	±	3	98	±	2	92	±	4
Other															
Methaqualone	90	±	2	94	±	0	96	±	2	96	±	4	93	±	1
Sildenafil	54	±	4	91	±	1	93	±	1	75	±	15	54	±	5
Drug precursors															
Ephedrine	94	±	7	86	±	1	80	±	0	82	±	3	88	±	1
Norephedrine	103	±	13	86	±	2	71	±	1	81	±	6	85	±	3

Table S7 – Standard deviation values in relation to mean values reported in Table 3

Compound	October									November								
	WWTP A			WWTP B			WWTP C			WWTP A			WWTP B			WWTP C		
	SPM	WW	%	SPM	WW	%	SPM	WW	%	SPM	WW	%	SPM	WW	%	SPM	WW	%
Stimulants																		
Cocaine	0.1	0.8	0.0	0.0	1.9	0.0	0.1	2.8	0.1	0.0	0.5	0.0	0.1	0.2	0.1	0.0	0.5	0.0
Benzoylecgonine	0.1	4.9	0.1	0.1	6.0	0.1	0.0	7.7	0.3	0.1	3.7	0.1	0.0	5.4	0.0	0.0	1.8	0.2
Norbenzoylecgonine	-	0.4	-	-	0.0	-	-	0.2	-	-	0.1	-	-	0.1	-	-	0.6	-
Norcocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cocaethylene	-	0.1	-	-	0.1	-	0.0	0.2	0.0	-	0.0	-	-	0.1	-	0.0	0.1	0.1
Anhydroecgonine M.E.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ecgonidine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amphetamine	0.2	13.1	0.3	2.9	2.2	0.2	7.4	16.1	1.4	-	1.8	-	-	26.1	-	0.5	39.6	0.2
Methamphetamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-
Methcathinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BZP	-	1.5	-	-	0.5	-	-	4.0	-	9.8	2.3	0.4	-	-	-	-	-	-
TFMPP	0.1	-	-	-	0.0	-	0.2	1.7	0.2	-	-	-	-	0.0	-	0.1	0.3	0.2
Hallucinogens																		
MDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDMA	-	0.0	-	-	0.3	-	0.1	0.2	0.1	0.0	0.3	0.3	-	0.2	-	0.1	1.1	0.1
MDEA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MBDB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BDB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mescaline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O-H-LSD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Opiods and morphine derivatives																		
Heroin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6-acetylmorphine	-	0.1	-	-	0.7	-	-	0.1	-	-	0.2	-	-	0.2	-	-	0.7	-
Codeine	3.0	35.6	0.0	4.5	33.9	0.1	16.2	66.5	0.1	0.4	2.7	0.0	5.4	7.7	0.1	16.1	32.6	0.1

Norcodeine	0.7	5.1	0.2	-	4.8	-	1.1	3.7	0.1	0.2	3.3	0.1	-	5.4	-	-	1.7	-
Oxycodone	-	0.0	-	-	0.3	-	-	0.3	-	-	0.6	-	-	0.9	-	-	0.2	-
Oxymorphone	-	-		-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-
Morphine	0.7	15.2	0.0	5.6	19.3	0.3	0.8	9.5	0.0	1.4	10.1	0.1	1.0	16.6	0.1	5.7	4.8	0.2
Normorphine	0.8	23.4	0.2	-	7.9	-	0.1	7.3	0.0	0.1	2.1	0.0	-	2.0	-	2.4	3.1	0.1
Dihydrocodeine	0.6	7.0	0.1	0.1	15.7	0.1	0.4	5.9	0.0	1.1	6.5	0.1	1.1	3.5	0.1	2.3	8.2	0.1
Buprenorphine	0.0	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	0.1	-	-
Norbuprenorphine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methadone	0.2	0.7	0.0	2.1	1.8	0.1	1.7	7.8	0.1	0.2	2.0	0.0	0.9	0.1	0.0	1.3	1.9	0.0
EDDP	1.7	1.8	0.0	1.8	4.6	0.0	12.3	1.7	0.1	3.7	0.5	0.1	3.2	1.0	0.1	6.4	0.2	0.0
EMDP	0.1	-	-	0.0	-	-	0.1	0.0	0.0	0.0	-	-	0.0	-	-	0.1	0.1	0.1
Fentanyl	-	-	-	-	-	-	0.1	0.1	0.2	-	-	-	-	-	-	-	-	-
Norfentanyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Propoxyphene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Norpropoxyphene	5.1	0.6	0.1	37.0	32.6	0.8	10.2	4.9	0.1	1.9	48.9	0.2	1.5	20.4	0.2	5.7	1.3	0.1
Tramadol	9.4	74.1	0.1	4.4	20.5	0.0	2.4	28.2	0.0	0.1	10.7	0.0	19.4	55.1	0.2	56.6	85.5	0.1
Nortramadol	11.7	60.6	0.3	27.4	59.7	0.3	112.4	68.8	0.6	3.3	16.3	0.3	34.5	66.1	0.2	32.7	45.2	0.1
Benzodiazepines																		
Temazepam	2.5	7.0	1.2	2.2	5.5	0.6	-	4.3	-	0.3	2.9	0.1	0.8	2.1	0.4	-	1.2	-
Diazepam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nordiazepam	-	8.8	-	-	3.9	-	-	2.5	-	-	1.1	-	-	7.7	-	-	5.9	-
Nitrazepam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7-aminonitrazepam	0.7	-	-	-	-	-	0.1	-	-	0.0	-	-	-	-	-	0.1	-	-
Oxazepam	-	0.1	-	-	0.8	-	0.5	1.6	0.1	-	0.4	-	-	0.4	-	0.5	1.0	0.1
Chlordiazepoxide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antidepressants																		
Dosulepin	2.1	1.4	0.0	34.8	7.5	0.5	22.3	3.0	0.1	4.9	13.5	0.1	22.3	2.7	0.3	7.9	8.2	0.1
Amitriptyline	9.5	0.4	0.0	56.2	40.7	0.5	14.5	3.4	0.0	7.3	21.7	0.1	26.1	7.8	0.2	2.2	25.9	0.0
Nortriptyline	0.5	0.0	0.0	2.4	0.8	0.6	4.7	1.4	0.1	1.4	3.0	0.2	2.6	0.3	0.4	0.3	1.1	0.0
Fluoxetine	4.8	1.8	0.1	2.9	2.9	0.1	10.7	2.5	0.1	8.0	1.3	0.1	0.7	0.8	0.0	11.9	2.6	0.1
Norfluoxetine	2.1	0.1	0.0	2.6	2.0	0.2	30.4	1.5	0.5	2.5	0.7	0.1	9.8	0.3	0.2	1.8	0.7	0.0
Venlafaxine	0.3	0.2	0.1	0.1	2.5	0.0	2.6	3.6	0.2	0.7	4.4	0.2	0.5	3.3	0.1	0.5	9.8	0.1

Dissociative anaesthetics																		
Phencyclidine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ketamine	0.3	1.4	0.1	0.2	1.2	0.1	0.2	0.4	0.1	0.2	1.1	0.1	0.0	0.1	0.0	0.7	3.0	0.1
Norketamine	-	0.8	-	-	-	-	-	0.7	-	-	-	-	-	0.4	-	-	0.6	-
Other																		
Methaqualone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sildenafil	0.9	0.0	0.1	1.1	2.3	0.3	2.5	0.3	0.2	1.5	0.3	0.2	2.3	0.4	0.2	0.0	1.2	0.1
Drug precursors																		
Ephedrine	-	34.2	-	-	79.2	-	-	203.6	-	-	15.2	-	-	31.1	-	-	15.2	-
Norephedrine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

REFERENCES

[1] A. Kaleta, M. Ferdig, W. Buchberger, Journal of Separation Science 29 (2006) 1662.
[2] A. Nieto, F. Borrull, E. Pocurull, R.M. Marcé, Journal of Separation Science 30 (2007) 979.
[3] L. Barron, J. Tobin, B. Paull, Journal of Environmental Monitoring 10 (2008) 353.
[4] K. Stein, M. Ramil, G. Fink, M. Sander, T.A. Ternes, Environmental Science & Technology 42 (2008) 6415.
[5] J. Radjenović, A. Jelić, M. Petrović, D. Barceló, Analytical and Bioanalytical Chemistry 393 (2009) 1685.
[6] P. Vazquez-Roig, R. Segarra, C. Blasco, V. Andreu, Y. Picó, Journal of Chromatography A 1217 (2010) 2471.
[7] C. Metcalfe, K. Tindale, H. Li, A. Rodayan, V. Yargeau, Environ. Pollut. 158 (2010) 3179.
[8] A.C. Moffat, D.M. Osselton, Widdop, Clarke's analysis of drugs and poisons, Pharmaceutical press 2004.
[9] ACD/I-lab, (version 12.0) accessed via ACD/chemsketch, Advanced chemistry development Inc. Toronto, ON, Canada. www.acdlabs.com,.
[10] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Ternes, W. Giger, Analytical Chemistry 73 (2001) 3632.
[11] A. Jelic, M. Petrovic, D. Barceló, Talanta 80 (2009) 363.